Inhibition of NO production in LPS-stimulated primary rat microglial cells by Bromelain

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Introduction:

Microglia, the sentries of the brain, are highly implicated in neurodegeneration as in neuroprotection. Chronic microglial activation endangers neuronal survival through the release of various potentially neurotoxic mediators including cytokines, chemokines and nitric oxide (NO). Thus, negative regulators of microglial activation have been considered as potential therapeutic candidates to target neurodegeneration, such as that in Alzheimer's, Parkinson's diseases and even in chronic epileptic syndromes. LPS is an endotoxin from Gram-negative bacteria, which provokes inflammatory and immunological responses.

Bromelain, a mixture of cysteine proteases, derived from pineapple stem (*Ananas comosus*), is known for its anti-inflammatory and immunomodulatory effects. Thus, we examined whether bromelain, as a natural plant extract, repress microglia activation and thereby confer neuroprotection against inflammation-related neuronal injury.

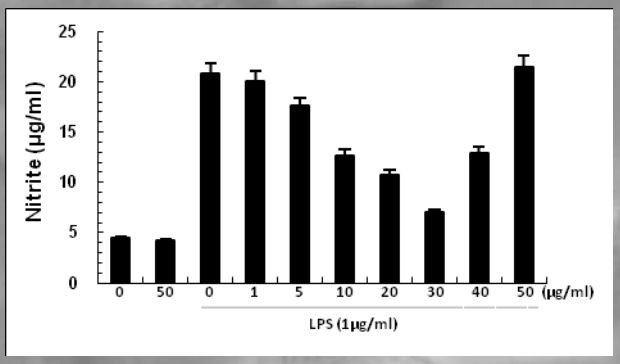
Methods and materials:

In the present study, we provided neonatal rat primary microglia to model inflammation in the brain. Primary microglial cells were prepared from cerebral cortices of one-day-old rat pups as described by McCarthy and de Vellis. After the cells became confluent at 12–14 days, the flasks were shaken to remove the microglia. Isolated microglial cells, cultured for 24 h, and the purity of the cultures was greater than 95% as judged by immunostaining with an anti-OX-42 antibody. The cells were pretreated with various concentrations of Bromelain (different doses between 1-50 mg/ml) in fresh medium containing 1% fetal bovine serum (FBS) for 30 min before lipopolysaccharide (LPS) (1mg/ml) treatments. Nitric oxide (NO) levels in the culture supernatants were measured by a Griess reaction.

Results:

Our results showed that Bromelain (30 mg/ml) significantly reduced the production of NO induced by LPS treatment in neonatal rat primary microglia in a dose-dependent manner (Fig. 1.A). Cell viability, using the MTT assay, was not reduced at the concentrations tested (Fig. 1.B)

A



B

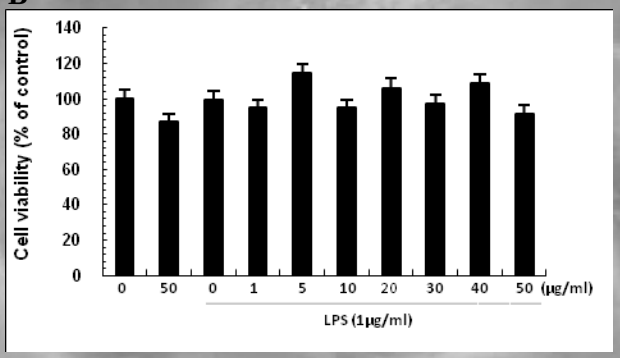


Fig. 1. Effect of bromelain on NO production in LPS-stimulated microglial cells. Primary microglial cells were incubated in the absence or presence of LPS (1 µg/ml). The cells were pretreated with various amounts of bomelain for 30 min before LPS was added. After 48 h, the cultures were subjected to a nitrite assay **(A)** and a cell viability assay **(B)**

Discussion and conclusion:

In conclusion, the anti-inflammatory effects of Bromelain in vitro suggest its potential effects in reduction of NO synthesis. So, as Bromelain has a natural origin, can be considered as a useful agent for neuroprotection or even reduction of symptoms of neurodegenerative pathologies.

Keywords: Microglia, Bromelain, CNS inflammation, NO, Neurodegeneration

References :

1. A
2. B
3. c