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Luteolin Attenuates LPS-Stimulated Pro-Inflammatory Molecule Expression and LPS-Induced Lethal Toxicity

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AIM

We have previously shown that luteolin (Lut) is one of the most efficacious flavonoids in inhibiting the expression of pro-inflammatory molecules by macrophages in response to LPS. In the present study we sought to investigate the molecular targets of Lut's actions and correlate inhibition of specific signaling molecules with the inhibitory action on TNF-α release. Exposure of RAW264.7 to LPS increased protein tyrosine, ERK1/2, p38 and Akt phosphorylation in a time-dependent manner. Pretreatment of cells with 10μM Lut for 30min inhibited the LPS-induced TNF-α release, as well as ERK1/2, Akt and p38 phosphorylation. Moreover, pretreatment with Lut inhibited IκB-α phosphorylation and degradation and NF-κB activation (p50/p65 nuclear translocation and NFκB-driven reporter gene expression). To test if the inhibitory action of Lut on TNF-α release is due to inhibition of Akt cells were transfected with a dominant negative form of Akt (K179M) prior to the LPS challenge. Such pretreatment did not reduce LPS-induced TNF-α release, suggesting that although LPS stimulates Akt phosphorylation in a Lut-sensitive manner, inhibiting Akt phosphorylation in not sufficient to block TNF-α release. Incubation of cells with the MEK1 inhibitor PD98059 (up to 10 μM) or the p38 inhibitor SB203580 (up to 2.5 μM) alone failed to inhibit LPS-induced TNF-α release, whereas a combination of SB203580 and PD98059 blocked it. These data taken together suggest that since luteolin blocks LPS-induced phosphorylation of both MAPK pathways, its ability to inhibit TNF-α production could result from the simultaneous inhibition of ERK1/2 and p38 pathways.

METHODS

To extend our findings *in vivo* and to test whether luteolin is capable of inhibiting LPS-induced lethal toxicity, we challenged mice with *Salmonella enteriditis* LPS with or without luteolin pretreatment.

RESULTS

LPS administration (32mg/Kg, i.p.) increased plasma TNF-α levels after 90min from undetectable levels to 11.0±2.5ng/ml serum. Pretreatment of mice with luteolin (0.2mg/kg, i.p.) reduced serum TNF-α levels to 1.1±0.2 ng/ml. Luteolin pretreatment also reduced LPS-stimulated intercellular adhesion molecule-1 expression in the liver and abolished leukocyte infiltration in the liver and lung. To determine if luteolin improves survival, mice were pretreated with luteolin or vehicle and were then challenged with LPS. After seven days only 4.1% of the mice receiving LPS remained alive. Thought the study, mice that had received luteolin showed an increased survival rate with 48% remaining alive on day 7.

CONCLUSIONS

We conclude that luteolin inhibits multiple LPS stimulated signaling pathways (protein tyrosin phosphorylation, ERK1/2, p38 and Akt phos phorylation as well as NF-κB activation), attenu ates pro-inflammatory molecule expression an protects against LPS-induced lethal toxicity i mice.