SL05 Kaurane diterpene inhibits NF-κB by targeting DNA-binding activity of p50 and blocks the expression of antiapoptotic and inflammarory NF-κB target genes

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Whole plants of *Isodon japonicus* (Labiatae) have been used in traditional medicine as a remedy for gastrointestinal disorders, cancer, and inflammatory diseases. Despite of its various pharmacological activities, the molecular mechanism of the plant has not been sufficiently explained. We isolated four kaurane diterpene compounds from the plant as an inhibitor of production of inflammatory mediators and NF-κB activation induced by LPS, indicating that these activities of them could explain, in part, *I. japonicus*'s diverse pharmacological activities such as anti-cancer and anti-inflammation. We investigated molecular mechanism of a major component kamebakaurin. Kamebakaurin prevented the activation of NF-κB by different stimuli such as LPS, phorbol esters, and TNF-α in various cell types. Treatment of cells with this compound prevent neither the induced degradation of IκB-α nor nuclear translocation of NF-κB by all stimuli. However, this compound significantly inhibited NF-κB activation, and interfered with DNA binding activity of active NF-κB in cell and *in vitro*. Furthermore, kamebakaurin preferentially prevented p50-mediated DNA-binding activity of NF-κB rather than that of RelA as measured using *in vitro* translated p50 and RelA proteins and a p50 mutant with Cys62Ser mutation. These results suggest that this compound exhibit its inhibitory activity by a direct modification of Cys62, which is critical for the DNA-binding activity of p50 subunit. Treatment of cells with kamebakaurin prevented the induced expression of anti-apoptotic NF-κB target genes such as c-IAP1 (hiap-2) and c-IAP2 (hiap-1), and Bfl-1/A1 by TNF-α, resulting in sensitizing MCF-7 cells to TNF-α-induced apoptosis. This compound also inhibited LPS-induced expression of inflammatory NF-κB target genes such as iNOS and COX-2 as well as the production of NO, PGE_2 and TNF-α in RAW264.7 cells, of which may correlated with the result of dose-dependent alleviation of inflammation in a *M. butylicum*-induced adjuvant arthritis model. Based on our results, kamebakaurin could serve as an interesting lead compound for the development of new, potent anti-inflammatory or anticancer agent. Furthermore, this study extends our understanding on the molecular mechanisms underlying the anti-inflammatory and anticancer activities of traditional medicinal plants, which contain kaurane diterpenoids abundantly.

References: 1. Hwang, et al. (2001) Planta Med., 67: 406-410. **2.** Lee, et al. (2002) J. Biol. Chem., 18411-18410.

SL06 Cytokine gene promoter-based *in vivo* screening for identification of novel anti-inflammatory and immunomodulatory herbal compounds/drugs

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In recent years there has been an increasing interest in the use of Chinese herbal medicines for the treatment of inflammatory and autoimmune diseases due to their reputed efficacy. It is desirable to understand the molecular mechanisms by which these medicinal herbs mediate their effects *in vivo*. Cytokines are inducible glycoproteins that play important regulatory roles in the immune system and are often used as therapeutic agents. Here we present a new approach that utilizes cytokine promoter-driven luciferase reporter-gene expression as a target for screening novel herbal drugs and evaluating their underlying molecular mechanisms *in vivo*. The promoter regions of important human pro-inflammatory cytokines such as TNF-α and GM-CSF were isolated, cloned into pGL-3 vector and the resultant plasmids were transfected into mouse epidermal tissues using a particle-mediated gene gun. The blasted skin was treated with crude extracts, solitary test herbal compounds or inflammatory agents. The naphthoquinones from *Lithospermum erythrorhizon* Sieb. & Zucc., in crude extract as well as the pure individual compounds shikonin, isobutyryl shikonin, acetyl shikonin, dimethylacrylic shikonin and isovaleryl shikonin showed significant dose dependent inhibition of TNF-α promoter activity induced by gene gun. The commercially available topical anti-inflammatory steroids hydrocortisone and betamethasone were also found to inhibit TNF-α promoter activity in our system. Croton oil, a well-known skin inflammation inducer, readily increased the transgenic GM-CSF-Lux promoter activity by 7-fold over the original control, whereas shikonin effectively decreased the GM-CSF promoter activity to 10-fold less than that of the inflamed controls. This investigation provides an *in vivo* system to understand the possible molecular basis for the therapeutic properties of traditional herbal medicines and also a molecular screening method to identify the novel therapeutic and/or immune modulatory agents for anti-inflammation and topical immunotherapy.