

Zyflamend[®] reduces LTB₄ formation, and may prevent oral carcinogenesis, in a DMBA-induced hamster cheek pouch model

Robert A. Newman¹, Peiying Yang¹, Diana Chan¹, Ning Li³, Zheng Sun⁴, and Xiaoxin Chen²

¹The University of Texas M. D. Anderson Cancer Center, Houston Texas; ²North Carolina Central University, Durham, NC; ³Institute of Nutrition and Food Hygiene, Chinese Center for Disease Control; and ⁴Beijing Hospital for Stomatology, Beijing, P. R. China

Background: Chronic inflammation has been proposed to contribute to carcinogenesis in many organ sites such as colon, lung and oral cancers. Aberrant arachidonic acid metabolism, especially COX and 5-LOX activities, have been associated with chronic inflammation as well as carcinogenesis in human oral cavity. Recently, Li *et al.* reported that the combination of two prescription medicines, Celecoxib (a COX-2 inhibitor) and Zileuton (a 5-LOX inhibitor), additively inhibited the incidence of squamous cell carcinoma in DMBA-induced hamster cheek pouch model (*Clin. Cancer Res. 11: 2089 – 2096, 2005*). Zyflamend is a combination of 10 concentrated herbal extracts each with potent anti-inflammatory activity. Since this product has shown inhibitory activity against both COX-1 and COX-2 as well as 5-LOX enzymes, it was of interest to investigate the effect of Zyflamend on development of DMBA-induced inflammation that is a precursor of oral squamous cell carcinoma.

Experimental Design: Hamster cheek pouch model was developed by topically applying 100 μ l of 0.5% DMBA onto the left cheek pouch of male Syrian golden hamster 3 times/wk for three weeks. After that, Zyflamend (25, 50, 100 μ l) was applied topically onto the left cheek pouch three times for one week. Tissues were then collected for analysis of histopathology and eicosanoids.

Results: After three treatments, Zyflamend significantly reduced infiltration of inflammatory cells, incidence of hyperplasia, number of dysplastic lesions as well as BrdU-labeling index in a concentration dependent manner (Table 1). When tissues were analyzed for the profile of eicosanoids using our newly developed LC/MS/MS analytical method, the level of LTB₄ in DMBA-treated tissues was increased 2-fold compared to control tissues. Interestingly, the formation of LTB₄ was reduced by 50% in the tissues treated with 100 μ l of Zyflamend ($P < 0.05$). The reduction of LTB₄ was concentration dependent as well.

Table 1. Inhibitory effect of Zyflamend on DMBA-induced oral lesions

Treatment group	Inflammatory cells/mm ²	No. of Hyperplasia	No. of Displasia	BrdU-labelling index
Negative control	10.4 \pm 4.7	1.17 \pm 1.61	0.67 \pm 1.15	0.67 \pm 0.40
Positive control	158.6 \pm 56.1	4.60 \pm 3.63	3.47 \pm 2.03	6.60 \pm 1.99
Zyflamend (25 μ l)	54.7 \pm 38.7***	2.31 \pm 0.94	2.10 \pm 1.78	5.55 \pm 2.28
Zyflamend (50 μ l)	30.9 \pm 8.6****	1.40 \pm 1.31	1.80 \pm 0.94	3.42 \pm 1.29**
Zyflamend (100 μ l)	46.5 \pm 26.7****	0.72 \pm 0.63*	1.03 \pm 0.97*	1.77 \pm 1.29****

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$ compared to the DMBA positive control group.

Conclusions: This study showed that Zyflamend inhibited LTB₄ formation and histopathological progression in the DMBA-induced hamster cheek pouch model. It suggests that Zyflamend may prevent oral carcinogenesis at the post-initiation stage, and its chemopreventive effect may partially depend on its inhibitory effects on LTB₄ formation. A long-term chemopreventive study on DMBA-induced hamster cheek pouch model is currently ongoing.