Cyclooxygenase (COX) is the critical enzyme involved in modulating inflammatory response through the synthesis of prostaglandins (Dannenberg et al. 2001). The inducible isoform of the enzyme, COX-2, is overexpressed in several malignant and premalignant lesions (Subbaramaiah et al. 1998; Elizabeth et al. 2002). Several preclinical and clinical studies have reported COX-2 inhibition as an effective strategy for chemoprevention. Nonsteroidal antinflammatory drugs (NASIDs) such as celecoxib, are the most widely investigated COX-2 inhibitors. The oil-soluble diallyl sulfides (DAS) include monosulfides (DAMS), disulfides (DADS) and trisulfides (DATS). They were found to be effective against canine and human tumors, the mechanism of which remains unresolved. We attempted a comparative evaluation of the antiproliferative effect of DAS in HEK 293T cells. The cells were treated with increasing concentrations of DAMS, DADS and DATS. There were significant differences between the IC50 values of DAMS, DADS and DATS. RT-PCR was performed and the expression of COX-2 was compared with that of β-actin. DATS inhibited COX-2 gene expression significantly stronger than DAMS and DADS. The data are suggestive of antineoplastic effect of DAS, mediated by controlling COX-2 expression.

**Key words**: anti-inflammatory, cyclooxygenase, COX-2, diallyl sulfides, garlic.

Cyclooxygenase (COX) is the critical enzyme involved in modulating inflammatory response through the synthesis of prostaglandins (Dannenberg et al. 2001). The inducible isoform of the enzyme, COX-2, is overexpressed in several malignant and premalignant lesions (Subbaramaiah et al. 1998; Elizabeth et al. 2002). Several preclinical and clinical studies have reported COX-2 inhibition as an effective strategy for chemoprevention, notably in colonic polyps and head and neck cancers. Treatment with selective COX-2 inhibitors reduces the formation of intestinal, esophageal, tongue, breast, skin, lung and bladder tumors in animals (Mestre et al. 1997; Subbaramaiah et al. 1998; Steinbach et al. 2000; Dannenberg et al. 2001). These studies indicate that COX-2 is a potential pharmacological target for anticancer therapy. Nonsteroidal antinflammatory drugs (NASIDs), such as celecoxib, are the most widely investigated COX-2 inhibitors (Elizabeth et al. 2002; Thun et al. 2002; Altorki et al. 2003). The long-term use of NASIDs in chemoprevention is associated with significant gastrointestinal toxicities. In this study we report on the effectiveness of diallyl sulfides (DAS), which are derivatives of garlic, as potent COX-2 inhibitors. When validated, they may serve as potential chemotherapeutic agents, possibly with better compliance than NASIDs.

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There are epidemiological studies reporting the association of increased garlic consumption with decreased incidence of cancers (Challier et al. 1998; Bianchini and Vainio 2001). In addition, experiments on animals have reported effectiveness of garlic extracts to inhibit a variety of tumors (Yang et al. 2001). Garlic contains a complex mixture of oil- and water-soluble organosulfur compounds. The oil-soluble DAS include monosulfides (DAMS), disulfides (DADS) and trisulfides (DATS). They were found to be effective against canine mammary tumor cells, murine melanoma cells, and human colon, lung, skin, neuroblastoma and prostate carcinoma cells (Sundaram et al. 1993; Takeyama et al. 1993; Sundaram et al. 1996; Sakamoto et al. 1997; Welch et al. 1992; Pinto et al. 1997). A dose-dependent effect of DAS over the cell cycle (G1 and S phases) was observed in human colon tumor cells (Knowles and Milner 1998).

In the present study we attempted a comparative evaluation of antiproliferative effect of DAS compounds in HEK 293T cells. The cells were treated with 5, 10, 20, 50 and 75 µM of DAMS, DADS and DATS for 24, 48 and 72 hours. The IC50 value of DAMS, DADS and DATS was found to be 30.0, 16.1 and 3.0 µM, respectively (data not shown). To elucidate the mechanism of antineoplastic effect, we analysed the gene expression of COX-2. Total RNA was isolated (TRIzol method) from treated and untreated (0 day) cells. The cDNA synthesized from the total RNA was used to amplify β actin and COX-2 mRNA (Elizabeth et al. 2002). The expression of COX-2 was compared with β actin – a housekeeping gene (Figure 1). The comparative analysis of the mRNA levels of COX-2 and β actin represented the action of the diallyl compounds over the regulation gene transcription. The normal level of β actin mRNA was observed in untreated and treated cells up to the concentration of 50 µM, but it diminished at 75 µM (for all the three compounds). With increasing concentrations, the cytotoxicity also increased, which may explain the decreasing levels of β actin mRNA. Significant amount of COX-2 mRNA was observed in untreated cells, which confirmed that in this cell line, the COX-2 gene is active. DAMS and DADS inhibited COX-2 expression at concentrations ≤ 10 µM after exposure for 24 and 48 hours. However, the effect was more pronounced in DATS, where the inhibition was seen even at 5 µM for the 24-hour treatment. With a longer treatment (72 hours), DAMS and DADS also inhibited COX-2 expression at 5 µM.

Promising preclinical findings and the encouraging results of a familial adenomatous polyposis study have led to several clinical trials using selective COX-2 inhibitors as a chemopreventive agent (Dannenberg 2001). A number of herbals with potential anti-inflammatory activity have been identified as the source of natural NSAIDs. Their use in cancer prevention may offer wide safety margins in terms of toxicity, as compared with pharmaceutically based NSAIDs (Wargovich et al. 2001). Garlic has known antithrombotic effects through fibrinolytic activity and reduction of platelet aggregation. Ingestion of garlic has also been reported to lower the concentration of triglycerides, cholesterol, and low-density lipoproteins, and to increase the concentration of high-density lipoproteins in blood (Bianchini and Vainio 2001).

We reported here on the potential anti-inflammatory activity of DAS (DAMS, DADS and DATS) derived from garlic by controlling the expression of COX-2. This suggests that DAS extracted from garlic may be used as potent COX-2 inhibitors. Molecular mechanisms of DAS-mediated COX-2 modulation need to be elucidated.

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