Development of anti-inflammatory and Anti-allergic Compounds from Medicinal herbs

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Mast cell play a crucial role in allergic and inflammatory disease through the exocytosis of preformed granule-associated mediators, such as histamine, serotonin, proteases and the generation of newly synthesized lipid mediators such as, leucotrien (LT) C_4 and prostaglandin (PG) D_2 in response to crosslinking of high affinity for IgE (FcγRI). New eicosanoids are synthesized by the oxidative metabolism of arachidonic acid, which is generally esterified *sn*-2 position of the major classes of glycerophospholipids. The first step of the eicosanoid generation involves the liberation of free arachidonic acid from the membrane phospholipids. This reaction is regulated mainly by two phospholipase A_2 (PLA_2) isozymes namely the 85-kDa cytosolic PLA_2 and 14-kDa secretory PLA_2. The second step leading to PGs generation involves cyclooxygenases (COXs) and LTs generation involves 5-lipoxygenase. There are two COX isoforms, COX-1 and COX-2, with similar molecular masses of approximately 72-KDa. COX-1 is constitutively expressed in most tissues and cells and is generally thought to serve certain physiological "house keeping functions", whereas COX-2 is dramatically induced in response to various stimuli, such as growth factors, cytokines or endotoxin, and mitogens, and is thought to contribute the generation of PGs in certain stages of cell proliferation and differentiation and at inflamed sites. The recent discovery of COX-2 led to the knowledge that selective inhibitors of this enzyme would constitute a novel approach to the treatments of inflammation with the diminished side effects. For this reason, many investigators have attempted to develop selective COX-2 inhibitors. Up to date, two distinct structural classes of

molecules have been reported as selective inhibitors of COX-2, including methanesulfonanalide class such as NS-398 and tricyclic inhibitor class such as Dup 697 and SC-57666.

During our continuous efforts to develop new anti-inflammatory and anti-allergic compounds from medicinal herbs, we isolated several compounds such as biflavonoid, prenylated flavone and iridoids using our *in vitro* (BMMC system) and *in vivo* assay systems. BMMC (bone-marrow derived mat cells) elicit a biphasic PGD_2–biosynthetic responses over time, the immediate and delayed phases of which depend entirely on COX-1 and COX-2, respectively, but not *vice versa.* This particular population of cells represents a useful tool for screening of new class of anti-inflammatory and anti-allergic compounds from various sources. Through the activity-guided isolation procedure, we successfully isolated selective COX-2 inhibitors, PLA_2 inhibitors and COX-2/5-LOX dual inhibitors. The present study describes how to develop anti-inflammatory and anti-allergic compounds from medicinal herbs.

MATERIALS AND METHODS

Preparation and activation of BMMC: Bone marrow cells from male BALB/c were cultured for up to 10 weeks in 50% enriched medium (RPMI 1640 containing 100 units/ml penicillin, 100 mg/ml streptomycin, 10 mg/ml gentamycin, 2 mM L-glutamin, 0.1 mM nonessential amino acids and 10 % fetal calf serum) and 50% WEHI-3 cell conditioned medium as a source of IL-3. After 3 weeks, >98% of the cells in the culture were BMMC. For cytokine stimulation, BMMC were washed once and suspended at a cell density of 1×10^6 cells /ml in enriched medium, and incubated for various periods with cytokines in the presence or absence of LPS at 37℃. After various periods of time, reactions were stopped by centrifugation at 120 g for 5 min at 4℃. The supenatants were retained for assay of mediator release, and the cells for analysis of the expression of mRNA and protein.

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Time course of PGD_2 generation and COX-2 induction by BMMC cultured with IL-10+IL-1β in the presence or absence of IgE/Ag