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Dual effects of acetylsalicylic acid on mast cell degranulation, expression of cyclooxygenase-2 and release of pro-inflammatory cytokines

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Abstract

Several studies have demonstrated that nonsteroidal anti-inflammatory drugs, such as acetylsalicylic acid (ASA), can have inhibitory or enhancing effects on inflammatory cell function. These effects seem independent of cyclooxygenase activity and prostaglandin synthesis inhibition. Here, we examined the effect of ASA on bone marrow-derived mast cells in more detail. ASA blocked the expression of cyclooxygenase-2, the production of tumor necrosis factor-α and interleukin-6, and the release of granule mediators from mast cells in a concentration-dependent fashion. Concomitantly, ASA inhibited nuclear factor (NF)-κB activity, as well as the phosphorylation and breakdown of the inhibitory protein IκB-α. We thus propose that the anti-inflammatory effects of ASA in mast cells are due to suppression of IκB kinase activity, thereby inhibiting subsequent phosphorylation and degradation of IκB-α, activation of NF-κB, and transcription of proinflammatory cytokines. The inhibition of BMMC degranulation was independent of NF-κB activation, however. Interestingly, the expression of cyclooxygenase-2 was not inhibited at 1 mM ASA, but was even enhanced significantly. The latter might contribute to the adverse effects of ASA in ASA-sensitive asthmatics.

Keywords: Mast cells; NF-κB; Allergy; Inflammation; IgE and antigen; ASA

1. Introduction

Acetylsalicylic acid (ASA) and its analogs are among the most widely used drugs on a worldwide basis. For more than three decades, the anti-inflammatory actions of ASA and salicylate have been attributed primarily to inhibition of prostaglandin synthesis [1]. However, a growing number of studies have provided evidence for immunomodulatory effects unrelated to inhibition of prostaglandin synthesis. For example, Casolaro et al. have reported that various NSAIDs such as ASA have dual effects on degranulation of human basophils and mast cells [2]. Moreover, a major finding was the discovery that ASA and salicylate specifically inhibit the activation of the transcription factor nuclear factor κB (NF-κB) by inflammatory stimuli [3]. NF-κB is an inducible transcription factor comprised of subunits that can include cRel, RelA, RelB, p50 and p52. In most cells the NF-κB prototype is a heterodimer composed of the RelA (p65) and NF-κB1 (p50) subunits. Cytoplasmic NF-κB is inactive when bound to an inhibitory protein IκB-α. NF-κB is activated by a wide variety of agents, including phorbol esters, IL-1β, TNF-α, lipopolysaccharide (LPS), double-stranded RNA, cAMP, bacteria, and viral transactivators [4,5]. Upon cell stimulation, the signal-responsive kinases IKKα and β are activated which directly phosphorylate Ser^{32} and Ser^{36} in IκB-α, triggering ubiquitination at Lys^{21} and Lys^{22}, and rapid degradation of IκB-α, in 26S proteasome [6]. This process liberates NF-κB, allowing it to translocate to the nucleus. In the nucleus, NF-κB binds to its cognate site, κB element, which regulates transcription of downstream genes. Transcription of most genes for inflammatory proteins, such as TNF-α, IL-2, IL-9, GM-CSF, and COX-2 is dependent on regulation by the transcription factor NF-κB in inflammatory or immune cells [4,7,8].

Abbreviations: ASA, acetylsalicylic acid; Anti-DNP IgE, anti-dinitrophenol IgE; COX, cyclooxygenase; DNP-HSA, dinitrophenol human serum albumin; HPRT, hypoxanthine-guanine phosphoribosyltransferase; MTT, 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide; NaSal, sodium salicylate; RT-PCR, reverse transcription polymerase chain reaction

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Mast cells and basophils are the main effector cells in IgE-mediated pathological symptoms seen in allergic disorders, such as urticaria, hay fever, and asthma [9,10]. Mast cells are also implicated in non-allergic acute and chronic inflammatory disease [11,12]. The signaling pathway leading to degranulation of mast cells after engagement of the FcεRI receptor by antigen and antibody has been extensively characterized [13,14]. Rapid activation of phosphorylation of intracellular target proteins, generation of IP3 and a subsequent increase in the cytosolic calcium level ($[Ca^{2+}]_i$) is considered to be essential in the mechanism of degranulation of mediators by mast cells [14]. The various cytokines and several bioactive substances, e.g. neuropeptides, complement, and kinins which are supplied from tissue microenvironments, are believed to act as positive or negative regulators of mast cell function [15]. Recently it has been shown that mast cells, through release of their pro-inflammatory mediators, are involved in aspirin attacks in aspirin-sensitive patients [16,17]. This prompted us to investigate the effects of ASA on the various functions of mast cells with respect to modulation of the NF-κB pathway.

2. Materials and methods

2.1. Materials

Recombinant mouse IL-1β, stem cell factor (SCF) and IL-10 were purchased from PeproTech. LPS (*Escherichia coli* 055.B5), Tyrode's salts and MTT were purchased from Sigma. RPMI 1640, fetal calf serum (FCS), and nonessential amino acids were purchased from GibCo BRL. Penicillin, streptomycin, 1-glutamine, sodium pyruvate, 2-mercaptoethanol, dinitrophenol-human serum albumin (DNP-HSA), ASA and sodium salicylate (NaSal) were obtained from Sigma. ASA was dissolved in deionized water at a concentration of 20 mM to be used as stock solution.

The ELISA kits for mouse TNF-α and IL-6 were purchased from Biosource. Rabbit polyclonal anti-IκBα and anti-p65 Abs were obtained from Santa Cruz Biotechnology. Rabbit polyclonal anti-phospho-IκBα Ab (Ser 32) was supplied by New England Biolabs. Goat anti-rabbit and rabbit anti-goat secondary Abs conjugated with horseradish peroxidase (HRP), were purchased from Dako. Proteasome inhibitor MG-132 (Z-Leu-Leu-Leu-H) and NF-κB SN50 were purchased from Calbiochem. Nuclear and cytoplasmic extraction reagents and Light Shift Chemiluminescent EMSA kits were purchased from Pierce. The NF-κB primer corresponding to NF-κB consensus sequence in the κ light chain enhancer in B cells (5′-AGT TGA GGG GAC TTT CCC AGG C-3′), end labeled with biotin and purified with HPLC, was purchased from Isogen Life Science.

2.2. Mouse bone marrow cultures

BMMC were generated from bone marrow of male BALB/cBy mice. Briefly, mice were sacrificed, and intact femurs were removed. Sterile endotoxin-free medium was repeatedly flushed through the bone shaft using a needle and syringe. The suspension of bone marrow cells was centrifuged at $320 \times g$ for 10 min, and cultured at a concentration of 0.5×10^6 nucleated cells/ml in RPMI 1640 with 10% FCS, 100 units/ml penicillin, 100 μg/ml streptomycin (Life technology), 10 μg/ml gentamycine, 2 mM L-glutamine and 0.1 mM nonessential amino acids (referred to as enriched medium). Pokeweed mitogen-stimulated spleen cell conditioned medium (PWM-SCM, see below) 20% (v/v) was added to the enriched medium. Flasks were then incubated at 37 °C in a 5% CO_2 humidified atmosphere. Nonadherent cells were transferred to fresh medium at least once a week. After 3–4 weeks, mast cell purity of >95% was achieved as assessed by toluidine blue staining.

2.3. Pokeweed mitogen-stimulated spleen cell conditioned medium (PWM-SCM)

Spleen cells from BALB/c mice (Charles River Breeding Laboratories) were cultured at a density of 2×10^6 cells/ml in RPMI 1640 medium containing 4 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.1 mM nonessential amino acids (complete RPMI 1640) containing lectin (8 μg/ml) and placed in 75-cm^2 tissue culture flasks. The cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere. After 5–7 days, medium was collected, centrifuged for 15 min at $3200 \times g$, filtered through a 0.22-μm Millipore filter, and used as PWM-SCM.

2.4. Survival assay

Viability of cells treated with ASA was determined by assaying the ability of mitochondrial dehydrogenases to convert a soluble tetrazolium, MTT, into an insoluble purple formazan by cleavage of the tetrazolium ring [18]. Briefly, cells were treated with 10 mM of ASA for 6 h. Then, the cells were washed, and their viability was measured by MTT assay as described previously [18].

2.5. Activation of BMMC

BMMC were washed once and suspended at a cell density of 10^7/ml in PWM-SCM 20% (v/v), and were sensitized with 10 μg/ml monoclonal anti-dinitrophenyl IgE for 45 min at 37 °C. After washing twice with enriched medium, cells were suspended at 2×10^6/ml in enriched medium containing SCF (100 ng/ml), IL-1β (5 ng/ml) and LPS (100 ng/ml). Then ASA or NaSal was added at