Saliicylic acid was identified in willow bark extracts as an active anti-inflammatory compound over a century ago. Because of its bitter taste, chemical derivatives of salicylic acid (2-hydroxybenzoic acid) were synthesized and tested. Acetylsalicylic acid (aspirin) eliminated the bitter taste but retained the anti-inflammatory action, and it was introduced for treating human maladies >100 years ago. It has remained the most commonly used drug for relieving pain, inflammatory symptoms, and fever. Aspirin also has established efficacy for preventing myocardial infarction and ischemic stroke, as well as for treating acute myocardial infarction. Furthermore, recent epidemiological studies indicate that aspirin use is accompanied by a reduction in cancers, especially colon cancer.

How does a simple compound exert such broad therapeutic actions? Vane and Botting discovered that aspirin and nonsteroidal anti-inflammatory drugs inhibit the synthesis of proinflammatory prostaglandin E2. Subsequently, others demonstrated that aspirin inhibits cyclooxygenase-1 (COX-1) activity by acetylating serine 530, which is located close to the active site (tyrosine 385 of COX-1), and that acetylation of this serine residue hinders the access of arachidonic acid to the active site. Aspirin inhibits COX-2 by a similar mechanism but is less potent because the substrate channel of the active site is larger and more flexible than that of COX-1. The therapeutic efficacy of aspirin in myocardial infarction and ischemic stroke has been clearly attributed to its inhibition of platelet COX-1 activity. In contrast, the mechanisms by which aspirin and salicylate exert their anti-inflammatory and antineoplastic actions are less clear.

Such actions were attributed to the inhibition of COX-2 activity, because COX-2 overexpression plays a key role in these disorders. This proposal is inconsistent with experimental and clinical findings. Aspirin has a short half-life in circulating blood (≈20 minutes) and is rapidly deacetylated and converted to salicylate in vivo. Salicylate does not affect COX-1 or COX-2 activity. Thus, the anti-inflammatory and antineoplastic actions of aspirin and salicylate remain a dilemma. Sodium salicylate paradoxically inhibited prostaglandin synthesis when added to intact cells. Furthermore, healthy subjects taking sodium salicylate excreted a significantly lower amount of prostaglandin metabolites in urine than those not taking sodium salicylate, and their levels of inhibition were comparable to those of patients taking aspirin and indomethacin. Aspirin also reduces human seminal prostaglandin levels. These data suggest that salicylate inhibits COX metabolism by a mechanism different from a direct inhibition of COX activity.

Xu et al recently reported that salicylate and aspirin block prostanooid synthesis in intact cells by suppressing COX-2 transcription in response to exogenous stimuli. Results from this report indicate that salicylate at therapeutic concentrations (10^{-5} to 10^{-4} mol/L) suppresses interleukin-1 and phorbol 12-myristate 13-acetate–induced expression of COX-2 mRNA and protein levels in serum-starved cultured human endothelial cells and fibroblasts. Its suppression of COX-2 expression occurs at the transcriptional level, as is evidenced by a concordant inhibition of nascent COX-2 mRNA synthesis and COX-2 promoter activity. Because COX-2 induction is a key event in inflammation and tumorigenesis, these findings provide an explanation for the in vivo effect of aspirin and salicylate.

Salicylate at higher concentrations (≥5 mmol/L) exhibits inhibitory actions on several transcriptional activators, especially nuclear factor (NF)-κB. NF-κB is bound to a family of inhibitor proteins called IκB and is sequestered in the cytoplasm. Cell activation results in IκB phosphorylation, which is catalyzed by IκB kinases. Phosphorylated IκB is dissociated from NF-κB and degraded by a ubiquitin-dependent mechanism. Free NF-κB is translocated into the nucleus, where it binds to its cognate sites present in proinflammatory genes and activates the transcription of these genes. Salicylate at suprapharmacological concentrations inhibits several steps in the NF-κB activation process, including (1) IκB kinase-β activity, (2) IκB phosphorylation, and (3) NF-κB–mediated gene transcription. Furthermore, salicylate inhibits ribosomal S6 kinase 2, which is also capable of phosphorylating IκBα in vitro. Several NF-κB dependent genes, such as those responsible for producing cytokines, intracellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin, are reportedly inhibited by salicylate. Salicylate at suprapharmacological concentrations, therefore, may suppress the inflammatory response through its inhibition of monocyte activation and of monocyte and granulocyte interaction with endothelial cells.

In this issue of Circulation, Marra et al provide insight into another action of salicylate. Their results demonstrate the...
inhibition of smooth muscle cell proliferation by salicylate and aspirin at 5 mmol/L. At this concentration, salicylate exerts broad actions on molecules intimately involved in cell cycle progression and control. Salicylate suppresses Rb phosphorylation and cyclin-dependent kinase 2 activity, as well as the level of cyclin A. However, it increases the levels of 2 key inhibitors of cyclin-dependent kinases, P21\textsuperscript{wdp} and P27\textsuperscript{kip1}, as well as P53. The authors also confirmed previous observations that salicylate at 5 mmol/L inhibits NF-κB activation in smooth muscle cells.

This study and several other reports have made interesting in vitro observations, but their pharmacological relevance in vivo is questionable, because the concentrations of salicylate and aspirin (≥5 mmol/L) that exert these effects on transcriptional and other cellular signaling pathways in vitro are toxic to human subjects. Furthermore, at high concentrations, such as those used in the in vitro experiments, salicylate exhibits nonspecific inhibition of a large number of kinases in cells, as was noted by Frantz and O’Neill. It is unlikely that these kinases and other signaling molecules will be a selective therapeutic target of salicylate. In contrast, salicylate at concentrations of 10^{-1} to 10^{-3} mol/L, which is commonly achieved by therapeutic doses of aspirin in humans, selectively blocks COX-2 transcription in fibroblasts, endothelial cells and macrophages.

Results from ongoing work in our laboratory have shown that COX-2 transcriptional suppression by salicylate is independent of NF-κB inhibition and due to the inhibition of CCAAT/enhancer binding protein-β (C/EBPβ) binding to the NF-interleukin 6 site located at the proximal region of the COX-2 promoter. The NF-interleukin 6–C/EBP binding site of the COX-2 promoter is required for the COX-2 transcription induced by a wide spectrum of stimuli. Therefore, C/EBPβ may be a specific target for salicylate at pharmacological concentrations.

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References


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