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Dossier: Aspirin

Aspirin induced apoptosis in gastric cancer cells

B.C.Y. Wong, G.H. Zhu, S.K. Lam

Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong, China

Summary – Aspirin has been well known for its anti-pyretic and anti-inflammatory action over the past century. Its main action in the gastro-intestinal tract has always been associated with erosion and ulceration. However in recent years, there has been evidence suggesting that aspirin and the more potent non-steroidal anti-inflammatory drugs (NSAIDs), could reduce the risk of gastric and colon cancer. One of the possible mechanisms in chemo-prevention is the ability to induce apoptosis in epithelial cells of the gastro-intestinal origin. This article introduces the role of apoptosis in the body and the gastro-intestinal tract. Evidence on the chemo-preventive role of aspirin and NSAIDs are listed, and the mechanisms of action discussed. © 1999 Elsevier, Paris

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Aspirin has clinical benefits related to its anti-platelet activity, anti-pyretic, and anti-inflammatory actions. It has also been known to be associated with gastro-intestinal side-effects, mainly in the form of gastric and duodenal ulcerations or erosions. However recently, there are data to suggest that aspirin is not always harmful to the gastro-intestinal tract, but instead is able to reduce the risk of gastric and colon cancer. The mechanism may be associated with alterations of the process called apoptosis in the gastro-intestinal epithelium. We present some of the recent evidence concerning the role of aspirin in gastro-intestinal cancer prevention and the underlying mechanism of apoptosis.

APOPTOSIS

Apoptosis, also known as programmed cell death, is a form of genetically regulated cell death under tight control. It is recognized and differentiated from necrosis by distinct morphological and biochemical features [1, 2]. This form of active cell death is found in all tissues, including gastro-intestinal epithelium, and can be induced by a wide variety of physiological and pathological stimuli. In the physiological state, there is a balance between proliferation and apoptosis. Cell loss via apoptosis does not produce inflammation, in contrast to cell death by necrosis, and therefore it does not induce any harm to the surrounding structures. Besides the regulation of physiological cell turnover, the important function of apoptosis is the removal of damaged cells with mutated DNA. This prevents the proliferation of malignant clones or the propagation of cells containing viral DNA [3]. Deregulation of the apoptosis pathway can lead to diseases (most notably cancers), auto-immune diseases, and neurodegenerative disorders [4, 5]. The role of apoptosis in a wide variety of diseases, together with its highly regulated nature, has made it an attractive new target for therapeutic intervention in diseases previously considered incurable, especially cancer.

Apoptotic cell death occurs in two phases: an initial commitment to cell death, followed by an execution phase characterized by morphological changes in cell structure. Cells of different organs, and more or less of different species, have common execution machinery. The external agents that trigger apoptosis include, but not exclusively, toxic chemicals, ultraviolet irradiation, chemotherapeutic agents, growth factor withdrawal, and tumor necrosis factors. These signals act through cell surface receptors or ligands and are transmitted to the cytosol and nucleus by signal transduction pathways. Activation of the caspase family begins the execution phase. Caspases are cysteine proteases which cleave critical cellular substrates and precipitate the dramatic morphological changes of apoptosis, first in mitochondria and the nucleus, and then followed by changes in the cell membrane.

NON-STEROIDAL ANTI-INFLAMMTORY DRUGS AND CYCLOOXYGENASES

Aspirin was the first synthetic non-steroidal anti-inflammatory drug (NSAID) to be commercialized. Together with other newer, more potent NSAIDs like indomethacin, they are used in a variety of clinical situations. It is generally accepted that aspirin and other NSAIDs exert their anti-inflammatory, anti-pyretic, and analgesic effects by the inhibition of cyclooxygenases (COXs), which catalyze the rate-limiting steps in the production of prostaglandins (PG) [6, 7].

So far at least, two cyclooxygenase isoforms have been identified, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). In 1976, Miyamoto et al. first purified COX-1 from bovine vesicular glands [8]. In 1989, Simmons et al. identified a second, inducible cyclooxygenase, known as COX-2 [9]. In many circumstances, COX-1 is constitutively expressed and exists in many tissues, including the stomach, kidney, and platelets. Its level does not fluctuate in response to stimuli such as cytokines or growth factors [7]. In contrast, COX-2 has a nearly undetectable low level in many tissues and its expression is increased in areas of inflammation [7]. COX-2 expression is regulated by a variety of agents, including growth factors, lipopolysaccharide, cytokines, and tumor promotors [10].

Most of the commonly used NSAIDs are non-selective inhibitors of cyclooxygenases. Aspirin irreversibly inactivates COX-1 through the acetylation of serine-530 [11], and alters COX-2 activity by the acetylation of serine-516 [12]. Indomethacin reacts with the active site of COX and induces a conformational change in the protein [13]. The ratio of IC50 for COX-2/COX-1 for aspirin and indomethacin is 166 and 60 respectively. Therefore, a drug that selectively inhibits COX-2 activity without affecting constitutive COX-1 activity might be efficacious in controlling inflammation without the side-effects commonly seen following the complete blockade of prostaglandin production [13, 14].

NSAIDS AND THEIR ANTI-NEOPLASTIC EFFECTS

The role of NSAIDs in cancer prevention was first explored in experiments in rodents in the late 1970s. NSAIDs, such as aspirin, indomethacin, piroxicam, and sulindac inhibit chemically induced tumors of the colon [15, 16], the esophagus [17], and other sites. Tumor growth often resumes when NSAIDs are discontinued [16]. In clinical studies, administration of sulindac reduced the number and size of colorectal polyps, precursors for colorectal carcinoma, in patients with familial adenomatous polyposis (FAP), an autosomal dominant disorder characterized by the formation of hundreds of colorectal adenomas and the development of colorectal cancer [18]. From these data, it has been hypothesized that the anti-tumor effects of NSAIDs were mediated by the inhibition of PG synthesis.

The earliest epidemiological observations regarding NSAID use and colorectal cancer was in 1988 as a retrospective case-control study. A 40–50% reduction in colon cancer incidence was seen among regular aspirin users [19]. Subsequent case-control studies confirmed this finding [20, 21]. Some evidence suggested that aspirin and NSAIDs may prevent other human gastrointestinal cancers in addition to colon cancers. In a large prospective study, Thun et al. examined fatal cancers in relation to aspirin among 635,031 adults who provided information on the frequency and duration of their aspirin use. Death rates decreased with more frequent aspirin use for cancers of the esophagus, stomach, colon, and rectum, but not generally for other cancers. For each digestive tract cancer, death rates were approximately 40% lower among those who took aspirin 16 times per month or more for at least one year compared to those who did not take aspirin. The trend of decreasing risk with more frequent aspirin use was strongest among people who took aspirin for ten years or more [22]. Record linkage studies in Finland and Sweden found a decreased risk of stomach and colorectal cancers, but not esophageal cancer in rheumatoid arthritis patients treated with high doses of aspirin and other NSAIDs [23, 24].

MECHANISMS FOR THE ANTI-NEOPLASTIC EFFECT OF NSAIDS

One potential mechanism for the anti-neoplastic effect of NSAIDs is the inhibition of PG synthesis. However, evidence suggested that sulindac sulfone, the oxidized form of sulindac that inhibits COX minimally, displayed the same chemo-preventive effect in rats as sulindac [25]; and salicylic acid, which had little COX inhibitory potency, inhibited the proliferation of several colon cell lines [26]. If NSAIDs exert their anti-proliferative effects by non-PG-dependent mechanisms, what are some of the possibilities for the mechanisms of their actions? Several investigations indicated that NSAIDs (including aspirin, sulindac and its active metabolite sulindac sulfide, indomethacin, piroxicam, and naproxen) inhibited cell division and altered the cell cycle phase distribution of colon cancer cells [27, 28].

NSAIDs inhibited cell proliferation by inducing cell cycle quiescence at the G_0/G_1 phase. This was achieved by decreasing the expression of cyclins B1 and E, and increasing the expression of cyclins D1, D2, and D3, particularly in the G_1 phase of the cell cycle and of the cyclin-dependent kinase inhibitor $p21^{waf1/cip1}$ [28]. We have previously shown that aspirin inhibited gastric cancer growth in a time- and dose-dependent manner, but unlike in colon cancer cells, this was not associated with alterations in cell cycle phase distribution [29]. Therefore, other mechanisms may be responsible for the growth inhibition of aspirin on gastric cancer cells.

APOPTOSIS INDUCTION

Induction of apoptosis is another mechanism by which NSAIDs exert their anti-neoplastic effects in the gastro-intestinal tract. Shiff et al. first reported that the NSAID sulindac and its metabolite sulindac sulfide, could increase the rate of apoptosis of cultured HT-29 cells in a dose- and time-dependent manner [27]. The examination was subsequently extended to include aspirin, indomethacin, naproxen, and piroxicam. All the drugs had similar effects on colon cancer cells. Piazza et al. [30] reported that sulindac sulfide as well as sulindac sulfone (the metabolite lacking anti-COX activity), induced apoptosis in HT-29 cells and several other normal and transformed cell lines from breast, melanoma, lung, and kidney. Sulindac sulfide was three- to four-fold as potent as sulfone. Apoptosis, rather than the inhibition of cell proliferation, was shown to be the mechanism for the growth inhibition of cells by sulindac sulfide and sulfone.

We previously reported that aspirin and indomethacin induced apoptosis in gastric cancer cells [29] in a time- and dose-dependent manner. Indomethacin has a more potent effect. Addition of PGE_2 did not inhibit aspirin induced apoptosis, suggesting that apoptosis was probably mediated by a non-PG related pathway, similar to the colon cancer scenario described above (unpublished data).

At present, there is an intensive effort to decipher the molecular pathways that mediate aspirin/NSAID-induced apoptosis. NSAID-induced apoptosis in vitro was not totally dependent on the p53 pathway, apoptosis could be stimulated in cells that contain wild-type p53, mutant p53, no p53 protein, or even a reduced level of mutant p53 [27]. We previously described a reduced sensitivity to indomethacin-induced apoptosis in gastric cancer cells with mutant p53 than in cells with the wild type p53 gene [31]. Apoptosis was associated with an increase in c-myc expression in the cells with wild type p53, but not in the cells with mutant p53. Upstream to the genetic expression, we have investigated the role of one signal transduction pathway, namely the protein kinase C (PKC) family. The PKC family are serine–threonine kinases involved in multiple stages of cell growth, differentiation, apoptosis, and neoplastic transformation. We found that activation of PKC using phorbol ester was able to rescue aspirin induced apoptosis [29], whereas consistent inhibition of PKC using specific pharmacological inhibitors alone was able to induce apoptosis [32]. We are currently investigating in detail the role of the PKC signal transduction pathway in aspirin induced apoptosis.

FUTURE DIRECTIONS

Induction of apoptosis by aspirin and NSAID in gastric and colon cancer leads to the exciting anticipation of using these compounds in chemo-prevention. Specific COX-2 inhibitors, which have a theoretical advantage of minimizing the gastro-intestinal side-effects of aspirin, receive wide attention. Its tumor suppressing effect has been established in vitro, but the effects on clinical human studies still need to be seen. Other derivatives such, as sulindac sulfone, have shown good anti-tumor effects in colon cancer. It may be a good candidate for gastric cancer as well. Hopefully, newer agents with no gastro-intestinal side-effects but potent anti-tumor effects will be available in the near future for gastric cancer prevention.

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