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Visions & Reflections (Minireview)

NSAIDs and apoptosis

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Abstract. Regular use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) has been associated with decreased incidence of cancer of the colon and other gastrointestinal organs. The chemopreventive properties of NSAIDs are due to their ability to induce apoptosis. Both COX-2-dependent and COX-2-independent mechanisms are involved in NSAIDs-induced neoplastic growth. This article reviews the recent literature that has revealed various important mechanisms of NSAIDs-induced apoptosis.

Keywords. NSAIDs, apoptosis, COX, colon cancer, proteasome, NF-κB.

Aspirin (acetyl salicylic acid) is a nonsteroidal anti-inflammatory drug (NSAID) that has long been used as an anti-pyretic and analgesic. It is also known to induce gastro-intestinal side effects primarily as gastric lesions, ulcerations and erosions [1]. All NSAIDs in general are used to treat pain, inflammation and fever. NSAIDs inhibit inflammation, mainly through their ability to inhibit cyclooxygenase (COX) activity [2]. COX is a key regulatory enzyme involved in prostaglandin (PG) biosynthesis, which is strongly implicated in the induction of inflammation [3]. This property makes NSAIDs valuable in treating various diseases associated with inflammation. Aspirin is also prescribed as a prophylactic treatment against coronary artery disease and stroke, most likely because of its inhibitory effect on the synthesis of thromboxane A2 in platelets [3]. Several epidemiological and experimental studies also revealed that the prolonged use of aspirin or other NSAIDs reduces the risk of cancer. The diverse effects of NSAIDs cannot be fully explained on the basis of their COX inhibition. Although the chemopreventive effects of aspirin and other NSAIDs remain unclear, both COX-dependent and -independent mechanisms might play an important role in the biological activity of NSAIDs. In this review, we particularly focus on recent understanding of the mechanisms of NSAIDs-induced apoptosis.

Apoptosis is a highly regulated form of cell death distinguished by the activation of a family of cysteine-aspertate proteases (caspases) that cleave various proteins, resulting in biochemical and morphological changes characteristic of this form of cell death. Extrinsic pathways involving the cell surface death receptor and intrinsic pathways involving mitochondria can induce apoptosis in tumor cells [4–6]. Specific drugs including NSAIDs and conditions can trigger apoptosis via the above-mentioned pathways [6].

Clinical, epidemiological and experimental evidence

Several clinical observations and epidemiological and experimental studies have found aspirin and other NSAIDs to be promising anti-cancer agents [7–20]. Prolonged use of NSAIDs has been reported to reduce the risk of cancer of the colon and other gastrointestinal organs as well as of cancer of the breast, prostate, lung and skin [7–20]. A recent systematic review and meta-analysis of nine observa-

tional studies assessing the association between NSAIDs use and the risk of gastric cancer suggested that long-term use of aspirin is associated with a significant, dose-dependent reduction in the risk of gastric and colon cancers [19]. The protective effect is seen for all age groups in both men and women [19]. Longer use associated with a lower risk, although the trend is not statistically significant. NSAIDs also have been associated with a reduced risk of sporadic large-bowel adenomas [16]. Evidence from both colorectal cancer and adenoma indicates that the protective effect of NSAIDs on neoplasia seems to require continuous use; infrequent use is not associated with reduced risk. NSAIDs also demonstrated anti-carcinogenic effects in experimental animal models [21–24]. Chemically induced gastric tumors in mice can be reduced by aspirin, ibuprofen, sulindac and indomethacin [21, 22]. NSAIDs also inhibit the growth of transplanted tumors and carcinogen-induced skin tumors in mice and rats [23, 24]. In both epidemiological and experimental findings, the anti-cancer effect of NSAIDs is reversible. The tumor recurrence increases shortly after discontinuation of the NSAIDs. Aspirin and other NSAIDs also inhibit cell proliferation and induce apoptosis in various cancer cell lines, which is considered to be an important mechanism for their anti-tumor activity and prevention of carcinogenesis [14, 25, 26]. A large body of evidence now suggests that both COX-2-dependent and COX-2-independent mechanisms are involved in NSAIDs-induced apoptosis [14, 25].

COX-2-dependent and -independent mechanisms

Two distinct isoforms of COX have been identified: COX-1 and COX-2. COX-1 is expressed constitutively in most mammalian tissues and involved in homeostatic functions such as maintenance of gastrointestinal mucosal integrity [27]. On the other hand, COX-2 is largely induced during inflammation [28]. Several studies have shown overproduction of COX-2 and PGE2 in gastric and colon neoplastic lesions, which suggests that the COX-2 overexpression is important during gastric carcinogenesis [29–31]. The anti-carcinogenic effect of NSAIDs therefore could be mediated via COX-2 inhibition. In fact, overexpression of COX-2 has been demonstrated to contribute to carcinogenesis by stimulating cell proliferation, inhibiting apoptosis and enhancing angiogenesis, and all of these effects are thought to be mediated via PGE2 [14, 25, 31]. Overexpression of COX-2 increases the cellular level of Bcl-2 and therefore may cause resistance to apoptosis of premalignant cells [32]. However, how COX-2 inhibition induces apoptosis is not well understood. Studies have suggested that decreased cellular PGE2 and increased arachidonic acid levels might be involved in the inhibition of cell proliferation and induction of apoptosis [26]. The increased cellular concentration of arachidonic acid can alter mitochondrial membrane permeability and cause cytochrome c release, leading to apoptosis [33, 34]. Arachidonic acid also increases the production of ceramide, a potent inducer of apoptosis [35]. COX-2 also has been shown to directly promote angiogenesis in several different experimental systems [36]. Angiogenesis is critical for progression of most human cancers [37]. Importantly, NSAIDs inhibit both COX-1 and COX-2 to varying degrees. The therapeutic effects of conventional NSAIDs are derived from inhibition of COX-2, while the adverse effects of these agents, particularly the upper gastrointestinal tract, arise from inhibition of COX-1 activity. This has led to the development of selective COX-2 inhibitors, which are comparatively less toxic to the gastrointestinal tract than the conventional NSAIDs [27,28]. However, it is important to note that some of the selective COX-2 inhibitors are toxic to the cardiovascular system.

Apoptosis caused by NSAIDs could not be fully explained by the COX inhibition. They were found to have anti-proliferative and apoptotic effects in cell lines, irrespective of their levels of expression of COX-1 or COX-2 [38]. Interestingly, NSAIDs could inhibit growth of colon cancer cell lines that do not express COX-1 and COX-2 enzymes and in mouse embryo fibroblasts that are null for both COX-1 and COX-2 [39, 40]. Most often, the doses of NSAIDs required to produce anti-carcinogenic effects and to induce apoptosis are relatively higher than to inhibit prostaglandin synthesis. The difference in the clinical activities of aspirin, at low and high doses, also led to speculation that not all the benefits of aspirin derive from inhibition of COX [41]. Several mechanisms have been proposed for NSAIDs-induced apoptosis, which are COX-2-independent. These include downregulation of nuclear factor-kappa B (NF-κB) activity [42–45], alteration in the levels of pro- and anti-apoptotic proteins [46–48], activation of extrinsic and intrinsic pathways of apoptosis [45, 49–52], inhibition of proteasome function [53,54], cell cycle arrest [53–57] and generation of stress response and activation of stress kinases [58–62]. Some of the above-mentioned apoptosis-induced mechanisms could also be regulated through COX-2-dependent pathways. However, NSAIDs differ in their ability to induce cell death, suppression of NF-κB activation and inhibition of expression of COX-2 [63,64]. Indomethacin, ibuprofen and sulindac were found to be the more potent inducers of apoptosis compared to aspirin [63,64].

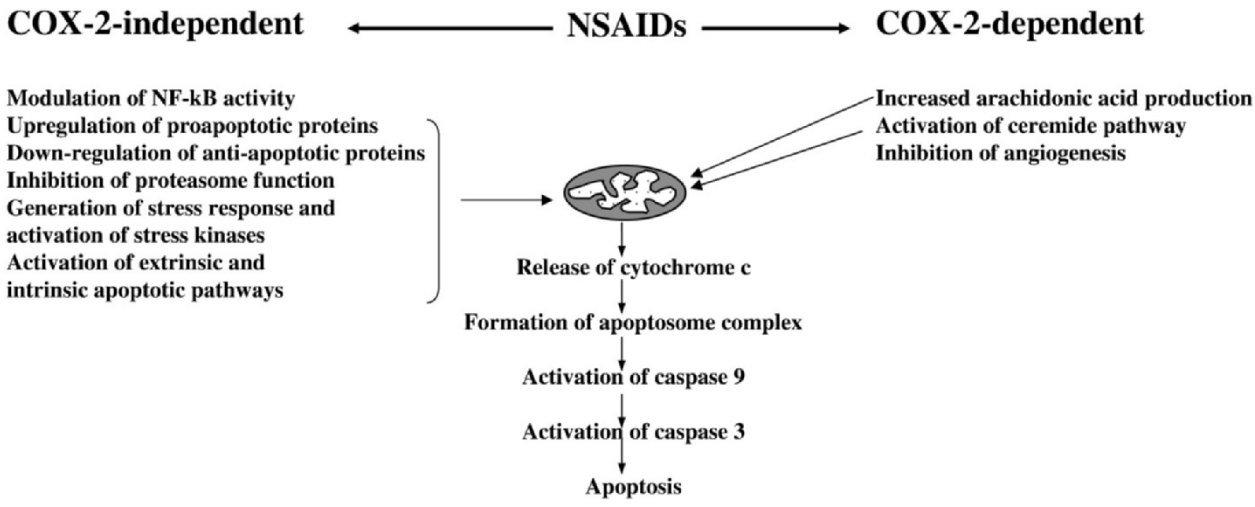

Figure 1. Potential mechanisms of NSAIDs-induced apoptosis.

Downregulation of NF-κB pathway

NF-κB is a ubiquitous factor that regulates the transcription of many genes involved in immune and inflammatory responses as well as cell survival and cell death [65–67]. NF-κB is localized in the cytoplasm in an inactive form in association with a family of inhibitory proteins called inhibitors of kappaB (IκBs). In response to multiple activating signals, IκB is phosphorylated and subsequently degraded by the proteasome. The rapid degradation of IκB proteins unmasks the nuclear localization signals of NF-κB, which then translocate to the nucleus and activate the transcription of multiple genes. Activation of NF-κB seems to stimulate some pathways that promote cell death, while other pathways promote cell survival [65, 66]. NF-κB activity is elevated in a variety of cancers, particularly advanced cancers that have been treated previously [67]. High NF-κB activity is also considered to be an important factor for the chemoresistance of many cancers [67, 68]. Several reports have suggested that aspirin and several other NSAIDs could promote apoptosis through the inhibition of NF-κB activity [42–45]. They also inhibit tumor necrosis factor-α (TNF-α)-mediated NF-κB activation and greatly sensitize gastric cancer cell lines to TNF-α [69]. In-depth studies have shown that NSAIDs inhibit IκB kinase β, an enzyme that activates the NF-κB pathway through phosphorylation and subsequent proteasomal degradation of IκB-α [42]. However, some reports have also demonstrated enhanced degradation of IκB-α and increased NF-κB activity in aspirin-induced apoptosis [70, 71]. Therefore, the effect of NSAIDs on NF-κB activity might be cell-specific and concentration-dependent. NSAIDs have also been reported to inhibit AP-1 activity, which might be linked with the induction of the apoptotic signal [61].

Activation of intrinsic and extrinsic apoptotic pathways

Aspirin and other NSAIDs have been found to induce apoptosis through mitochondrial pathways by cytochrome c release and activation of caspase-9 and extrinsic pathways by activation of caspase-8 [47, 49–52]. Release of cytochrome c from mitochondria is a central event in apoptosis [72]. It has also been demonstrated that cytochrome c release from mitochondria is an early event in NSAIDs-induced apoptosis [49, 53]. Cytochrome c released into the cytosol can bind with the apoptotic protease activating factor-1 (apaf-1) and form the apoptosome complex, which in turn leads to the sequential activation of caspase-9 and caspase-3. Aspirin-induced apoptosis can be partially prevented by caspase-8 and caspase-3 inhibitors and overexpression of the anti-apoptotic protein Bcl-2 [47, 49, 50]. apaf-1-deficient cells are very much resistant to aspirin-induced apoptosis [49]. Activation of caspase-8 could also play an important role in aspirin-induced apoptosis [47]. Activated caspase-8 could amplify the apoptotic signal either by directly activating downstream caspases or by cleaving the BH3 interacting domain death agonist (Bid). Cleaved tBid can translocate to the mitochondria and induce cytochrome c release.

Upregulation of pro-apoptotic proteins and down-regulation of anti-apoptotic proteins is also a possible target for NSAIDs-mediated apoptosis [46–48]. NSAIDs have been shown to downregulate Bcl-2-expression and induce the expression of Bcl-2 asso-

ciated X protein (Bax), Bcl-2 antagonist of cell death (Bad) and p53 [46–48, 53]. Bax could translocate from the cytosol to the outer mitochondrial membrane and make pores in the membrane to release cytochrome c [73]. Downregulation of Bcl-2 could further promote the release of cytochrome c. Cytochrome c release from mitochondria can also be induced by arachidonic acid and ceremide, which are increased during COX-2 inhibition. Therefore, mitochondria could be one of the common targets of both COX-2-dependent and independent apoptotic pathways induced by NSAIDs. NSAIDs have also been reported to downregulate the expression of protein kinase C-β1 (PKC-β1) [74]. Since PKC-β1 acts as a survival mediator, its downregulation could potentially induce cell death.

Inhibition of proteasome function and cell cycle arrest

NSAIDs also could induce cell cycle arrest and apoptosis through inhibition of proteasome function [53–57]. The ubiquitin proteasome system (UPS) is the cell's major extralysosomal pathway responsible for intracellular protein degradation in eukaryotes. This pathway is involved in the degradation of several critical regulatory proteins associated with regulation of the cell cycle and differentiation [75,76]. It functions through a two-step system. First, the target proteins are attached to a chain of ubiquitin molecules through covalent attachment and then, in the second step, the tagged protein is recognized and degraded by the proteasomal machinery. Since the UPS is involved in the degradation of many short-lived proteins that are required for cell survival, it is expected that the dysfunction of this pathways will promote cell death. Indeed, this has been proven true by the several reports showing that pharmacological inhibition of proteasome function induces dual apoptotic signaling pathways, depending on cell types and conditions [77–79]. Several studies have also shown that proteasome inhibition generates stress response and induces various heat-shock proteins, which increase cell tolerance to stressful conditions [80]. Proteasome inhibitors also inhibit NF-κB activity, induce oxidative and endoplasmic (ER) stress, and activate various stress kinases. Treatment of aspirin in various cell lines has been found to decrease proteasome activity and increase accumulation of ubiquitylated proteins, which correlates with its effect on cell death [53]. Aspirin and other NSAIDs exposure also increases the intracellular accumulation of various proteasomal substrates which are pro-apoptotic, like Bax, IκB-α, p53, $p21^{waf1/Cip1}$ and $p27^{kip1}$ [46, 47, 53, 55, 56]. Increased accumulation of $p27^{kip1}$ or $p21^{waf1/Cip1}$ would result in cell cycle arrest at the G1/S phase and apoptosis. But how aspirin or other NSAIDs induce proteasomal malfunction is not known. However, NSAIDs-induced proteasomal dysfunction could explain diverse reported effects of NSAIDs, including inhibition of NF-κB activity, cell cycle arrest, and induction of dual apoptotic signaling pathways and stress response. All these effects can be observed during proteasomal inhibition.

Induction of stress response

NSAIDs have been found to induce ER and oxidative stress, and these stress responses could be involved in the initiation of apoptotic signals [58–60]. ER stress response is induced when unfolded proteins accumulate in the ER. If this stress response is insufficient, the apoptotic response is initiated by both activating transcription factor (ATF) 4- and 6-dependent activation of C/EBP homologous transcription factor (CHOP). ER stress also activates apoptosis signal-regulating kinase 1 (ASK1), and activated ASK1 induces apoptosis through cJun N-terminal kinase (JNK) [81]. ER stress also causes activation of caspase-12. Activated caspase-12 can further activate caspase-9 and -3 [81]. Indomethacin has been shown to induce both glucose-regulated protein-78 and CHOP [60]. It also causes activation of ATF6, ATF4 and X box binding protein-1, ASK1 and JNK [60–62, 82]. NSAIDs-induced apoptosis could also be initiated through the generation of oxidative stress, which activates the intrinsic pathway of apoptosis involving mitochondria [58, 59]. Generation of ER or oxidative stress by NSAIDs also most likely depends on the concentrations and the types of drugs used [83]. Nitric oxide-donating NSAIDs, which are safer than their NSAIDs counterpart, are more potent inducers of oxidative stress and apoptosis [83].

Conclusion

It is very clear from the literature that multiple pathways are associated with aspirin and other NSAIDs-induced apoptosis. Some of those pathways are COX-2-dependent while others are COX-2-independent. Further understanding of the mechanisms of NSAIDs-induced apoptosis will greatly help in designing more potent and safer drugs for the treatment of gastric and colon cancers. One of the major problems of NSAIDs is that they require higher doses to produce anti-carcinogenic effects and to induce apoptosis. Selective COX-2 inhibitors or nitric oxide-donating NSAIDs with minimum gastrointestinal side effects could be promising anti-cancer drugs.

They can also be combined with drugs targeting other oncogenic pathways for further improvement of clinical outcome.

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