

Potency of non-steroidal anti-inflammatory drugs in chemotherapy (Review)

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Abstract. Cancer cell resistance, particularly multidrug resistance (MDR), is the leading cause of chemotherapy failure. A number of mechanisms involved in the development of MDR have been described, including the overexpression of ATP-dependent membrane-bound transport proteins. The enhanced expression of these proteins, referred to as ATP-binding cassette (ABC) transporters, results in an increased cellular efflux of the cytotoxic drug, thereby reducing its intracellular concentration to an ineffective level. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most frequently consumed drugs worldwide. NSAIDs are mainly used to treat pain, fever and inflammation. Numerous studies suggest that NSAIDs also show promise as anticancer drugs. NSAIDs have been shown to reduce cancer cell proliferation, motility, angiogenesis and invasiveness. In addition to these effects, NSAIDs have been shown to induce apoptosis in a wide variety of cancer types. Moreover, several studies have indicated that NSAIDs may sensitise cancer cells to the anti-proliferative effects of cytotoxic drugs by modulating ABC transporter activity. Therefore, combining specific NSAIDs with chemotherapeutic drugs may have clinical applications. Such treatments may allow for the use of a lower dose of cytotoxic drugs and may also enhance the effectiveness of therapy. The objective of this review was to discuss the possible role of NSAIDs in the modulation of antitumour drug cytotoxicity. We particularly emphasised on the use of COX-2 inhibitors in combination with chemotherapy and the molecular and cellular mechanisms underlying the alterations in outcome that occur in response to this combination therapy.

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1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are primarily used as analgesics, antipyretics and anti-inflammatory agents. NSAIDs mainly act by inhibiting prostaglandin (PG) production. A number of experimental, epidemiological and clinical studies have revealed the antitumour properties of NSAIDs, particularly of cyclooxygenase (COX)-2 inhibitors (1,2). NSAIDs have been shown to inhibit malignant transformation in several cancer cell lines. Moreover, the frequent use of NSAIDs has been associated with a reduced risk of colorectal, gastrointestinal, breast, prostate and lung cancer (3-6). The mechanism underlying the antitumour activity of NSAIDs has not been fully elucidated; however, it may involve the inhibition of COXs or other non-COX enzymatic pathways.

2. NSAIDs

The use of herbal extracts containing salicylates dates back thousands of years. In 1874, Maclagan successfully used salicylic acid isolated from willow bark for the treatment of the inflammation associated with rheumatic fever (7). A more effective and tolerable synthetic acetylated form of salicylic acid was introduced by Felix Hoffman in 1897; this derivative was named aspirin (8,9). Over time, several other drugs with the same antipyretic, analgesic and anti-inflammatory properties were introduced, including antipyrine, acetaminophen, phenylbutazone, naproxen and indomethacin. As these drugs share a similar mechanism of action and are clearly distinct from other groups of drugs used in the treatment of inflammation (glucocorticoids), they were collectively named non-steroidal anti-inflammatory drugs (NSAIDs) (10,11).

The main mechanism through which NSAIDs exert their effects is the inhibition of PG biosynthesis. PGs have been implicated in a number of physiological and pathological disorders, such as inflammation, pain, pyrexia, cancer, osteoporosis, cardiovascular diseases and asthma (12,13). Following exposure to physiological and pathological

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stimuli, polyunsaturated fatty acids, including arachidonic acid (AA), are released from membrane phospholipids through the action of phospholipase A2 enzymes. Free AA is subsequently converted via one of three enzymatic pathways (14-16) (Fig. 1): In the COX pathway, AA is converted to PGs, prostacyclins (PCs) and thromboxanes (TXs); in the lipoxygenase (LOX) pathway, AA is converted to hydroxyeicosatetraenoic acids (HETEs), leukotrienes (LTs) and lipoxins (LXs); lastly, in the cytochrome P450 (CYP450) monooxygenase pathway, AA release leads to the production of HETEs and epoxyeicosatrienoic acids (EETs). Additionally, in a non-enzymatic pathway, AA release results in the synthesis of isoprostanes. The products of these metabolic pathways are referred to as eicosanoids. Eicosanoids represent important intercellular and intracellular signalling molecules that participate in a wide range of physiological processes, such as the regulation of smooth muscle tone, vascular permeability, platelet aggregation, transporter proteins and proliferation. In addition, eicosanoids are involved in inflammation, autoimmunity, angiogenesis, allergic diseases and cancer (17-20). Extensive research has been focused on PGs and other COX-derived metabolites. However, a number of studies suggested that LOX-derived products also affect the development and progression of several malignancies (21-25).

COXs and their inhibitors in cancer treatment. There are 3 COX isoforms, commonly referred to as COX-1, COX-2 and COX-3. COX-1, also referred to as PGH synthase, is the key enzyme responsible for the oxidation of AA to PGG₂ and PGH₂. COX-1 is constitutively expressed, with its levels remaining constant under most physiological and pathological conditions. By contrast, the expression of COX-2 is highly inducible in response to mitogenic and inflammatory stimuli, such as fibroblast growth factor (26), transforming growth factor β (27), epidermal growth factor (28), vascular endothelial growth factor, tumour necrosis factor α and interleukins 1 α and 1 β (29). The function of COX-3 remains unclear (30-32). An aberrant constitutive expression of COX-2 has been demonstrated during the early stages of carcinogenesis (33,34). There is compelling evidence supporting a role for COX-2 in tumour development. COX-2 expression has been shown to be elevated in several human tumours, including colorectal (35,36), gastric (37) and pancreatic cancer (38), oesophageal adenocarcinoma (39), lung (40) and breast cancer (41). The tumour-promoting effect of COX-2 may be a consequence of the numerous effects that COX-2 exerts on cells. COX-2 may promote proliferation, angiogenesis and invasiveness, prevent apoptosis and enhance cell adhesion and motility (42). Treatment with COX-2-specific inhibitors results in a wide range of cellular effects, including induction of apoptosis, reduction of cell proliferation, inhibition of angiogenesis and enhanced anticancer drug-induced cytotoxicity (43-46). These findings suggest that NSAIDs may exert their anticancer effects through COX-2 inhibition. Although the significance of COX-2 inhibitors is well established, the mechanism underlying their chemopreventive and chemotherapeutic actions is largely unknown. Indeed, there is evidence suggesting that the antitumour effect of NSAIDs may not only be mediated by the inhibition of COX-2 activity, but that other cellular targets may also play a role (46). This

hypothesis is supported by the observation that NSAID treatment reduced cell survival in COX-2-overexpressing as well as COX-deficient cancer cell lines (47-49).

LOXs and their inhibitors in cancer treatment. Information regarding the role of LOXs in the promotion of cancer growth is limited. The identification of LOX isoforms in cancer, stromal and immune cells has led to the hypothesis that these enzymes may contribute to tumour development and growth (50), with interest mainly focused on 5-LOX, 12-LOX and 15-LOX. Under physiological conditions, the expression of 5-LOX is limited to immune cells (51,52). 5-LOX may directly control tumour cell function or indirectly affect the tumour microenvironment. Increased 5-LOX activity has been demonstrated to play a role in the early stages of colon cancer (53) and in carcinogenesis in human oral cavity tissues (54). It was also reported that 5-LOX expression may be involved in the development of BCR-ABL-induced chronic myeloid leukaemia (55). Moreover, the 5-LOX pathway may be involved in the metastatic process of pancreatic, intestinal and prostate cancers (56,57). The inhibition of 5-LOX expression and activity promotes cell apoptosis and tumour growth arrest. Additionally, 5-LOX inhibition affects epithelial-to-mesenchymal transition in certain cancer cell lines and suppresses metastasis in pancreatic cancer. These effects are likely due to the upregulation of E-cadherin and paxillin (58-61). The finding that 12-LOX is overexpressed in murine lung carcinoma and human prostate cancer cells suggests a possible role for this enzyme in cancer development (22,62). The 12-LOX inhibitor baicalein induces apoptosis in cancer cells. This induction is mediated through the regulation of the B-cell lymphoma-2 (Bcl-2) protein (63-65). Furthermore, 12-LOX controls G1/S-phase arrest by inhibiting Akt and mitogen-activated protein kinases and regulating the expression of nuclear factor (NF)- κ B (66). A proangiogenic function for 12-LOX products has also been suggested. The downregulation of 15-LOX expression has been shown in breast and prostate cancer and colorectal adenocarcinomas (67-70). The 15-LOX-2 isoform suppresses cell cycle progression and promotes cell senescence (70-72). Taken together, these findings suggest that LOXs may be potential targets for anticancer therapy.

P450 monooxygenases and their inhibitors in cancer treatment. CYP450s are monooxygenases that catalyse a variety of reactions. These enzymes have variable substrates, including fatty acids, steroids and xenobiotics. CYP450 enzymes are localised to the mitochondria and the endoplasmic reticulum. Mitochondrial CYP450s metabolise endogenous substrates, whereas microsomal CYP450s are involved in the metabolic reactions of exo- and endogenous substrates. Significant attention has been focused on the roles of COX- and LOX-derived products in carcinogenesis; however, little is known regarding the role of CYP450-derived products in this process. CYP450 activity in cancer cells may lead to the deactivation of antitumour drugs, thereby limiting therapeutic efficacy. The CYP1, CYP2 and CYP3 families are important enzymes that metabolise a significant number of clinically important drugs (73). Aberrant CYP450 enzymatic activity has been detected in a variety of human cancer cell lines and has been shown to contribute to neoangiogenesis, cancer cell migration, tumour growth and metastasis (74-78).

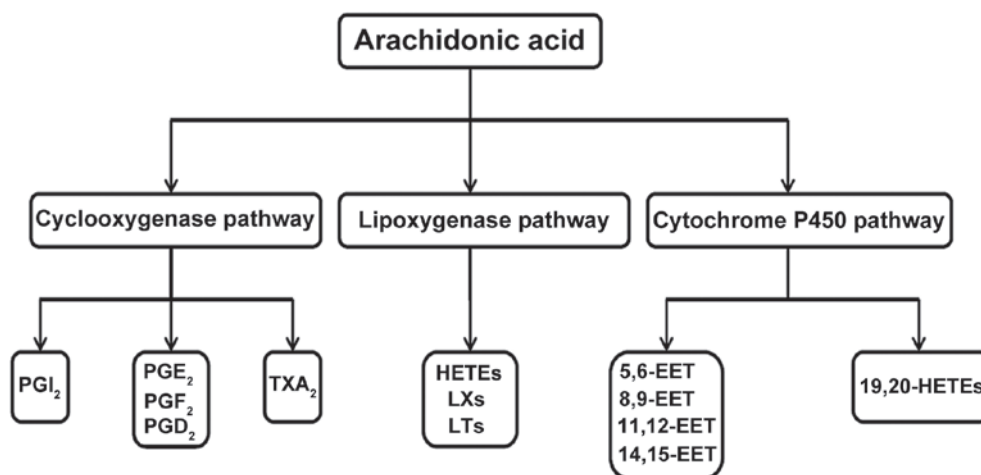


Figure 1. Enzymatic arachidonic acid metabolism. PG, prostaglandin; TX, thromboxane; HETE, hydroxyeicosatetraenoic acid; LX, lipoxin; LT, leukotriene; EET, epoxyeicosatrienoic acid.

3. NSAIDs in cancer treatment

Drug resistance is considered to be a major hindrance to the success of chemotherapeutic treatment. Multidrug resistance (MDR) is a multifactorial phenomenon and is often associated with the overexpression of ATP-binding cassette (ABC) transporter proteins (79,80). Accumulating evidence indicates that NSAIDs exert a chemosensitising effect; however, the exact mechanism underlying this action remains unknown, although several molecular mechanisms have been suggested.

Combination of NSAIDs with chemotherapeutic drugs in vitro. NSAIDs, particularly COX-2 inhibitors, may suppress MDR by inhibiting ABC transporters and sensitise cancer cells to the antiproliferative effects of anticancer drugs. These effects of NSAIDs have been demonstrated in several different malignancies (81-85). Permeability glycoprotein (P-gp), which acts on a broad substrate range, is one of the most extensively investigated and best characterised transporter proteins. NSAIDs have been shown to suppress the expression and function of this transporter in a variety of cancer cell types. Zattelli *et al* (85) demonstrated that treatment with the selective COX-2 inhibitor NS-398 resulted in significantly increased doxorubicin accumulation and sensitivity in chemoresistant MCF7 breast cancer cells. Those effects depended on the inhibition of P-gp expression and function. By contrast, it was suggested that NSAIDs are not involved in the regulation of P-gp activity and function and that their chemosensitising effect is mediated through different mechanisms (86). However, the majority of the studies contradict this hypothesis. Awara *et al* (87) reported an enhancement of doxorubicin anti-tumour activity with celecoxib-induced P-gp inhibition. This was demonstrated by a significant reduction in the efflux of the P-gp substrate Rhodamine 123. Similar findings were reported by other research groups (82,85,88,89). Indomethacin and a COX-2 selective inhibitor, SC236, sensitised HepG2 human hepatocellular carcinoma cells to the cytotoxic effects of doxorubicin. This effect was the result of increased intracellular retention and accumulation of doxorubicin via the inhibition of P-gp and MDR associated protein 1 (MRP1) expression

and activity (90). Kang *et al* (91) detected an inhibition of the MRP1 efflux pump and enhanced doxorubicin cytotoxicity with celecoxib treatment. Similar results were obtained by Ko *et al* (92), where celecoxib not only reverted MRP1-related drug resistance, but also inhibited the function of breast cancer resistance protein (BCRP). Due to its expression in malignant hematopoietic and lymphoid cells, BCRP potentially plays an important role in drug resistance, not only in breast cancer, but also in hematological malignancies. Furthermore, BCRP is expressed in leukaemic stem cells, contributing to the resistance of these cancers to chemotherapy or targeted therapy (93). The drugs used to treat these cancers are often BCRP substrates. Little is known regarding the effects of NSAIDs on antitumour drug cytotoxicity in hematological malignancies. Accumulating evidence indicates a positive effect of NSAIDs on chemotherapeutic drug action in BCRP-overexpressing solid tumours. Co-treatment with mitoxantrone and indomethacin sensitised resistant MCF-7/MX cells to mitoxantrone (94). Studies that combined NSAIDs with cisplatin-based chemotherapy have yielded opposing results. A recent study revealed that celecoxib and SC-236 antagonised the cytotoxicity of cisplatin in human gastric cells, whereas indomethacin and nimesulid exerted no effects (95). By contrast, the use of another COX-2 selective inhibitor, JTE-522, in combination with cisplatin, resulted in synergistic antitumour activity in a gastric cancer cell line (96). In other cancer cell lines, celecoxib potentiated the cytotoxicity of cisplatin (97,98). The discrepancy regarding the effects of NSAIDs on cisplatin action may be partially explained by the different chemical structures of the utilised NSAIDs and by the different tumour cell types employed (95).

Apart from ABC transporter inhibition, other mechanisms have been suggested to explain the chemosensitising effect of NSAIDs, including the inhibition of several transcriptional factors, varying functions of COX-2 in cancer cells, ceramide production and DNA hypermethylation (Table I). NF- κ B inhibition may play a role in NSAID-enhanced anti-tumour drug cytotoxicity (99). NF- κ B has been shown to be involved in chemoresistance in different cancer types. The constitutive expression of this transcription factor in tumours

Table I. Effects of NSAIDs on cytotoxic drug efficacy in different cancer cell lines.

NSAIDs/chemotherapeutics	Cancer cell line	Effect	Mechanism of action	Author (Refs.)
Celecoxib/doxorubicin	ECC	Syn	Inhibition of P-gp	Awara <i>et al</i> (87)
	H460	Syn	Inhibition of MRP1	Kang <i>et al</i> (91)
	MDA-MB-231	Syn	Inhibition of NF- κ B	van Wijngaarden <i>et al</i> (99)
	MCF7	Ant	DNA damage repair	El-Awady <i>et al</i> (107)
Celecoxib/vincristine	KB/VCR	Syn	Inhibition of P-gp	Yan <i>et al</i> (88)
Celecoxib/imatinib	K562	Syn	Inhibition of P-gp	Arunasree <i>et al</i> (82)
Celecoxib/cisplatin	TMK1	Ant	Enhanced efflux, reduced influx	Chen <i>et al</i> (95)
	MCF7	Ant	DNA damage repair	El-Awady <i>et al</i> (107)
Celecoxib/5-FU	MCF7	Ant	DNA damage repair	El-Awady <i>et al</i> (107)
JTE-522/cisplatin	MKN-45	Syn	Inhibition of ABC	Sugiura <i>et al</i> (96)
Celecoxib/tamoxifen	MCF7	Syn	Inhibition of P-gp due to DNA methylation	Xia <i>et al</i> (100)
NS-398/doxorubicin	MCF7	Syn	Inhibition of P-gp	Zatelli <i>et al</i> (85)
Indomethacin, SC-236/doxorubicin	HepG2	Syn	Inhibition of P-gp, MRP1	Ye <i>et al</i> (90)
Indomethacin/mitoxantrone	MCF7/MX	Syn	Inhibition of BCRP	Elahian <i>et al</i> (94)

Syn, synergistic effect of combinatory therapy; ant, antagonistic effect; ECC, Ehrlich carcinoma cell line; H460, non-small-cell lung carcinoma cell line; MDA-MB-231 and MCF7, breast carcinoma cell lines; MCF7/MX, mitoxantrone-resistant breast carcinoma cell line; KB/VCR, oral cancer cell line; K562, erythromyeloblastoid leukaemia cell line; TMK1 and MKN-45, human gastric adenocarcinoma cell lines; HepG2, hepatocellular carcinoma cell line; 5-FU, 5-fluorouracil; P-gp, permeability glycoprotein; MRP, multidrug resistance protein; NF, nuclear factor; ABC, ATP-binding cassette; BCRP, breast cancer resistance protein.

protects against apoptotic stimuli. Moreover, the inhibition of NF- κ B activity may affect intracellular drug accumulation and transport. The enhanced accumulation of doxorubicin in MDA-MB-231 human breast cancer cells upon celecoxib treatment was not mediated by changes in COX-2 enzyme activity or through P-gp, MRP1 or BCRP inhibition, but rather due to the inhibition of NF- κ B. Xia *et al* also demonstrated that NSAIDs may sensitise cancer cells to antitumour drugs by inducing DNA hypermethylation (100). The ability of celecoxib to modulate DNA methylation has also been demonstrated (101). The expression of the *MDR1* gene, which codes for the P-gp protein, is regulated through the methylation of CpG islands located within the *MDR1* promoter (102-104). Xia *et al* observed that treatment with celecoxib significantly enhanced CpG island methylation, which led to the suppression of P-gp expression (100). The ability of celecoxib to repress the activity of the transcription factor Sp1 was previously demonstrated (105). The *MDR1* gene promoter contains a binding site for this factor. This binding site may be susceptible to celecoxib-induced hypermethylation, thereby limiting the ability of Sp1 to bind DNA. Celecoxib, in combination with the 5-LOX inhibitor MK-886, exerted a significant additive cytotoxic effect on Caco-2 and HT-29 cancer cells, which was, in part, mediated by ceramide-induced apoptosis (106). El-Awady *et al* (107) demonstrated the diverse effects of celecoxib on the anticancer activity of etoposide, cisplatin, 5-fluorouracil (5-FU) and doxorubicin in five cancer cell lines,

namely the HeLa, HCT-116, HepG2, MCF7 and U251. In the MCF7 breast cancer cell line, the interaction of celecoxib with these four chemotherapeutics was antagonistic, indicating that celecoxib is of little value when used in combination with anti-tumour drugs in the treatment of breast cancer. By contrast, other data indicate that celecoxib enhances the cytotoxicity of anticancer drugs in breast cancer cells (99,108). The interaction of celecoxib with etoposide, cisplatin and 5-FU was shown to be dependent on the cancer cell line employed, the drug type used and the incubation schedule. The combination of celecoxib and the same antitumour drug also exerted different effects on different cell lines. One plausible explanation for this finding may be that COX-2 has different roles in different cancer types (107). In cancers where COX-2 increases tumour growth and progression (109), COX-2 inhibitors may be of therapeutic benefit. However, in other malignancies, COX-2 has been reported to exert proapoptotic and tumour-suppressing effects (110-112). In such cancer types, COX-2 inhibition may lead to enhanced tumour growth, inhibition of apoptosis and decreased efficacy of anticancer drugs (107). Several studies reported a direct association between COX-2 expression and the ABC transporters P-gp and MRP1. Patel *et al* (113) demonstrated that the overexpression of COX-2 led to increased P-gp expression and activity, whereas the COX-2 inhibitor NS398 was able to block this increase. In colon cancer, a resistance to cisplatin resulted from COX-2 overexpression, which induced MRP1 expression (114). A positive correlation

Table II. Effects of NSAIDs on cytotoxic drug efficacy in animal models.

NSAIDs/chemotherapeutics	Experimental model	Effect	Mechanism of action	Author (Refs.)
Celecoxib/doxorubicin	Mouse SEC	Syn	Inhibition of P-gp	Awara et al (87)
	Rat SH-SY5Y	Syn	Inhibition of angiogenesis and cell proliferation	Ponthan et al (127)
Celecoxib/irinotecan	Mouse MCA	Syn	Unknown	Trifan et al (139)
	Rat SH-SY5Y	Syn	Inhibition of angiogenesis and cell proliferation	Ponthan et al (127)
Celecoxib/5-FU	Mouse colon 26 cells	Syn	Inhibition of tumour growth and angiogenesis	Irie et al (125)
	Mouse HT-29	Syn	Cytochrome c-dependent apoptotic pathways	Zhang et al (129)
Piroxicam/cisplatin	Dog TCC	Syn	Unknown	Knapp et al (126)
	Mouse MSTO-211H	Syn	Enhanced expression of intracellular drug effectors	Spugnini et al (128)
Aspirin/doxorubicin	Mouse HepG2	Syn	Reduction of tumour growth and weight	Hossain et al (124)
JTE-522/docetaxel, vinorelbine	Mouse ACC-LC-319	Syn	Inhibition of tumour growth	Hida et al (123)

Syn, synergistic effect of combinatory therapy; SEC, solid Ehrlich carcinoma; SH-SY5Y, neuroblastoma cell line; HT-29, human colorectal cancer cell line; MCA, mouse colorectal adenocarcinoma; colon 26 cells, colon cancer cell line; TCC, transitional cell carcinoma; MSTO-211H, human mesothelioma cell line; HepG2, human hepatocellular carcinoma cell line; ACC-LC-319, human lung adenocarcinoma cell line ; 5-FU, 5-fluorouracil; P-gp, permeability glycoprotein.

between the expression of COX-2 and P-gp was also reported by studies on hepatocellular carcinoma, breast and ovarian cancer (115-117). COX-2 was found to be involved in the regulation of P-gp, MRP1 and BCRP transporter expression via the COX-2/PGE2/PGE receptor 4/phosphatidyl inositol 3-kinase pathway (116,118).

Synergistic effects of NSAIDs with hypericin (HY)-mediated photodynamic therapy (PDT) have also been reported (119-122). The specific inhibition of COX, LOX and CYP450 activity increased the efficacy of HY-PDT in the HT-29 cancer cell line (121). An important role for the MRP1 and BCRP transporters in HY efflux was also demonstrated (119). Proadifen, a P450 monooxygenase inhibitor, was shown to inhibit these transport proteins, resulting in a significant increase in intracellular HY accumulation in HT-29 cells and MRP1 and BCRP-overexpressing cells.

Taken together, the abovementioned findings indicate that the mechanism through which NSAIDs affect the action and effectiveness of cytotoxic drugs varies. The exact mechanism may depend on the cancer cell line, the structures of the NSAIDs and chemotherapeutics, the specific interactions between the drugs and the incubation schedule. The mechanism underlying the NSAID-induced increase in antitumour drug cytotoxicity may be one of the abovementioned processes. However, more than one mechanisms are likely involved.

Combination of NSAIDs with chemotherapeutic drugs in vivo. A growing amount of evidence from various animal models suggests positive effects of NSAID use in combination with antitumour drugs (87,123-129) (Table II). However, the exact

mechanism through which this combined treatment results in improved antitumour activity in *in vivo* models is not clearly understood. Given the complexity of animal models in comparison to *in vitro* systems, the effects of the tumour microenvironment, tumour angiogenesis, the immune system and pharmacokinetic processes must be taken into consideration (123,126,130). As NSAIDs may alter ABC transporter expression or activity in cancer cell lines, this mechanism may also be involved *in vivo*. Awara *et al* (87) reported that the inhibition of P-gp activity by NSAIDs is likely responsible for the enhanced antitumour effects of doxorubicin. It was suggested that NSAIDs exert their growth-inhibitory functions and synergistic effects with chemotherapeutics through multiple pathways. Neoangiogenesis plays a key role in tumour promotion and progression. Certain studies demonstrated the ability of NSAIDs, particularly selective COX-2 inhibitors, to suppress tumour growth by inhibiting angiogenesis and cell proliferation (131,132). Although the suppression of angiogenesis that occurs with NSAID treatment alone may not be sufficient to inhibit tumour growth, NSAIDs may enhance the antiangiogenic and antiproliferative effects of certain antitumour drugs (123,125,127). As shown by Irie *et al* (125), celecoxib alone did not significantly inhibit tumour growth, although it did exhibit a certain antiangiogenic activity. However, in combination with 5-FU, celecoxib enhanced the antitumour effect of 5-FU and significantly suppressed angiogenesis and tumour growth, likely via the inhibition of VEGF and the induction of IFN- γ (125). Treatment with celecoxib in combination with doxorubicin and irinotecan was also found to be effective in decreasing tumour growth

Table III. Effects of NSAIDs on cytotoxic drug efficacy in clinical trials.

NSAIDs/chemotherapeutics	Cancer type	Effect	Mechanism of action	Author (Refs.)
Celecoxib/paclitaxel, carboplatin	NSCLC	Syn	Inhibition of COX-2	Altorki <i>et al</i> (152)
Celecoxib/docetaxel	NSCLC	None	-	Schneider <i>et al</i> (144)
	NSCLC	Syn	Inhibition of COX-2	Nugent <i>et al</i> (153)
Celecoxib/FOLFIRI	CRC	None	-	Maiello <i>et al</i> (143)
Celecoxib/FOLFIRI, CAPIRI	CRC	None	-	Kohne <i>et al</i> (142)
Celecoxib/platin derivatives, gemcitabine, vinorelbin	NSCLC	None	Various COX-2 expression levels	Koch <i>et al</i> (151)
Celecoxib/carboplatin, docetaxel	NSCLC	None	-	Groen <i>et al</i> (150)
Celecoxib/carboplatin	OC	Syn	Potential inhibition of COX-2	Legge <i>et al</i> (154)
Celecoxib/transtuzumab	BC	None	-	Dang <i>et al</i> (148)
Rofecoxib/5-FU	CRC	None	-	Becerra <i>et al</i> (147)

Syn, synergistic effect of combinatory therapy; NSCLC, non-small-cell lung carcinoma; CRC, colorectal adenocarcinoma; OC, ovarian cancer; BC, breast adenocarcinoma; SCLC, small-cell lung carcinoma; FOLFIRI, leucovorin + 5-FU + irinotecan; CAPIRI, capecitabine + irinotecan; COX, cyclooxygenase; 5-FU, 5-fluorouracil; -, inadequate dosing.

through the inhibition of cell proliferation and the suppression of tumour vasculature (127). A number of intracellular signalling proteins are involved in cell proliferation, survival and apoptosis. Several lines of evidence suggest that COX-2 may elevate the levels of the antiapoptotic proteins Bcl-2 and Mcl-1 through mitogen-activated protein kinase activation, which results in an inhibition of the cytochrome *c* pathway (133-135). Moreover, a study by Zhang *et al* (129) revealed an improved therapeutic benefit of 5-FU via celecoxib addition, which occurred through the induction of the cytochrome *c*-dependent apoptotic pathway, as well as a possible role for 5-FU in the celecoxib-mediated inhibition of COX-2 expression. As previously mentioned, the antiproliferative, antiangiogenic and antitumour effects of NSAIDs may be, to a certain extent, COX-2-independent. Consistent with these findings, piroxicam was able to exert its effect via a COX/PGE₂-independent mechanism (128). Moreover, piroxicam enhanced cisplatin-induced cytotoxicity via the upregulation of endogenous drug effectors and the inhibition of certain cell growth regulators. *In vitro* studies demonstrated that NSAIDs may mediate their antitumour effects through modulation of the NF- κ B signalling pathway (99,136). NF- κ B, with its dual anti- and proapoptotic functions, plays an important role in regulating cellular proliferation and apoptotic cell death. The inhibition of NF- κ B activity may be responsible for the celecoxib-induced doxorubicin cytotoxicity that results in decreased tumour volume (99). By contrast, certain studies suggested that NSAIDs may activate NF- κ B, thereby inducing apoptosis (137,138). Apart from enhancing the cytotoxicity of chemotherapeutic drugs, the addition of NSAIDs may also reduce the severity of chemotherapy-associated adverse effects, such as late diarrhoea and cachexia (139).

Combination of NSAIDs with chemotherapeutic drugs in clinical trials. Due to the limited effectiveness of certain cancer treatments, it is necessary to establish a novel treat-

ment strategy that improves patient response to chemotherapy. A large number of studies have demonstrated that COX-2 may be involved in the development of several cancer types. COX-2 may positively affect multiple processes, including tumour cell growth, migration and invasiveness, but may also downregulate apoptosis and angiogenic stimulation (35-38). Moreover, the overexpression of COX-2 may also reduce the response of cancer cells to cytotoxic therapy (140). Preclinical studies suggested that treatment with NSAIDs, particularly COX-2 inhibitors, may affect the outcome of chemotherapy through various mechanisms, including the inhibition of neoangiogenesis and the induction of apoptosis (130,131,141). Despite promising preclinical results with NSAIDs in combination with antitumour drugs, little is known regarding the effects of this combination on humans. The currently available clinical results are contradictory and mainly disappointing (142-145) (Table III). For example, several combinations did not appear to improve therapy outcome, including celecoxib and docetaxel (144,146); celecoxib and 5-FU (142,143); rofecoxib, 5-FU and leucovorin (147); celecoxib and transtuzumab (148); rofecoxib, cisplatin and gemcitabine (149); celecoxib, docetaxel and carboplatin (150); and celecoxib and platinum derivatives (151). However, certain phase II studies have yielded encouraging results. In the case of non-small-cell lung carcinoma (NSCLC), the combination of celecoxib and chemotherapy was associated with increased overall survival (152,153). In the case of heavily pretreated recurrent ovarian cancer, the administration of celecoxib in combination with carboplatin-based chemotherapy also yielded promising results (154). The discrepancy in various results may be due to multiple factors, such as complex pharmacodynamic interactions between NSAIDs and the cytotoxic drugs and the varying levels of intratumoural COX-2 and ABC transporters (155-159). The role of COX-2 expression in the response of cancer cells to combined NSAID and antitumour drug therapy was demonstrated by Edelman *et al* and

has been supported by other studies (155,160). Patients with COX-2-overexpressing tumours who did not receive combined celecoxib/chemotherapy treatment exhibited a significantly worse outcome. Possible adverse effects in patients with COX-2-non-expressing tumours that received celecoxib treatment were also demonstrated (160).

4. Conclusion

NSAIDs are potent antitumour drugs, capable of inhibiting tumour angiogenesis, proliferation, invasion and motility, as well as of inducing apoptosis. Furthermore, a number of experimental and preclinical studies indicated that combining NSAIDs with antitumour drugs may improve outcome. The exact mechanism underlying this synergistic effect has not yet been fully elucidated, but may involve several diverse processes, including the inhibition of COX-2 expression, ABC transporter activity or NF- κ B. However, the results of combined therapy in clinical trials are mainly disappointing. Despite significant efforts to determine the exact mechanism through which NSAIDs modulate the efficacy of anticancer drugs, there remain several unanswered questions.

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