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Celecoxib enhances doxorubicin-induced cytotoxicity in MDA-MB231 cells by NF-κB-mediated increase of intracellular doxorubicin accumulation

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) and cyclo-oxygenase (COX) inhibitors are anti-inflammatory agents that have also shown to be useful in anticancer therapy. In the present study, we show that the specific COX-2 inhibitor celecoxib enhances the inhibitory effect of doxorubicin (dox) on human MDA-MB231 breast tumour growth *in vivo* and *in vitro*. We also found that celecoxib increased the intracellular accumulation and retention of dox *in vitro*. Since the NSAID indomethacin and the specific COX-2 inhibitor NS398 did not affect the *in vitro* actions of dox, these effects are likely to be mediated *via* a COX-independent mechanism. It has been suggested that some COX-inhibitors can enhance the actions of cytostatics by overcoming multidrug resistance through the inhibition of ABC-transporter proteins. However, we found that the three main ATP-binding cassette (ABC)-transporter proteins, implicated in dox transport, were inactive in MDA-MB231 cells. Therefore, the finding that the P-glycoprotein (P-gp) blocker PSC833 also increased cellular accumulation of dox was unexpected. In order to unravel the molecular mechanisms involved in dox accumulation, we examined the involvement of NF-κB, as this transcription factor has been implicated in celecoxib action as well as in chemoresistance. We found that celecoxib and PSC833, but not indomethacin or NS398, almost completely inhibited basal- and dox induced NF-κB gene-reporter activity and p65 subunit nuclear translocation. Furthermore, the NF-κB inhibitor PDTC mimicked the actions of celecoxib and PSC833 on cell growth and on intracellular accumulation of dox, suggesting that NF-κB is functionally involved in the actions of these compounds. In conclusion, we show that structurally different compounds, among which are celecoxib and PSC833, increase the intracellular accumulation of dox and enhance dox induced cytotoxicity in MDA-MB231 breast cancer cells most likely via the modulation of NF-κB activity.

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1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) and specific cyclo-oxygenase (COX)-2 inhibitors are widely used in the treatment of pain and rheumatoid arthritis and have shown promising results in the treatment of cancer in experimental and clinical studies.[1,2] COX-2 is overexpressed in many malignancies and is involved in tumour development and growth. The effects of NSAIDs and specific COX-2 inhibitors on tumour cells include inhibition of cell proliferation, induction of apoptosis and reduction of cell motility and adhesion.[2–10] Furthermore, both non-specific and specific COX-2 inhibitors have shown to significantly inhibit tumour angiogenesis.[11–14] These anticancer properties make it worthwhile examining the possible benefit of combining NSAIDs and COX-2 inhibitors with conventional anticancer therapies, such as chemotherapy.

Several preclinical and clinical studies have explored and are currently exploring the therapeutic benefit of combining NSAIDs and specific COX-2 inhibitors with chemotherapeutics and have been shown to improve treatment outcome. For example, in experimental and clinical studies, the COX-2 inhibitor celecoxib has shown to enhance the anti-tumour efficacy of several cytostatics, such as that of irinotecan, doxorubin (dox), bleomycin and 5-fluorouracil.[15–18] The mechanism by which COX-2 inhibitors enhance the action of cytostatics is, however, not clear and it is suggested that this may involve mechanisms other than suppression of the COX-2 enzyme. For example, it has been proposed that COX-inhibitors modulate the resistance of tumours to chemotherapeutic drugs by affecting the activity of plasma membrane transporter proteins of the ABC-transporter family, which behave as energy-dependent efflux pumps for cytostatics. The three key mammalian transporters involved in the transport of anticancer agents, such as the anthracyclines, are P-glycoprotein (P-gp/ABCB1), multidrug-resistance protein-1 (MRP1/ABCC1) and breast cancer resistance protein (BCRP/MXR/ABCG2).[19,20]

In recent years, much effort has been made to identify agents that are able to overcome MDR, in order to improve chemotherapeutic treatment. These agents, called chemosensitisers, belong to a variety of structural classes, such as calcium channel blockers, drug analogues, cyclic peptides and steroids.[21,22] It has been suggested that COX-inhibitors may also act as chemosensitisers and can overcome MDR by inhibiting P-gp[15] or MRPs.[23–26] However, conclusive evidence for the actions of NSAIDs and specific COX-2 inhibitors on these transporters is lacking. Interestingly, apart from MDR, NF-κB has recently been described as another COX independent molecular target for actions of NSAIDs and COX-2 inhibitors, such as aspirin, indomethacin and celecoxib.[27–30] Moreover, NF-κB has also shown to be involved in chemoresistance in different cancer types[31–36], suggesting a possible role of this transcription factor in the chemosensitising effect of COX-inhibitors.

In the present study, we addressed these issues both *in vivo* and *in vitro* by studying the effects of NSAIDs, specific COX-2 inhibitors such as celecoxib and specific pump inhibitors in combination with dox in the breast cancer cell line MDA-MB231.

2. Materials and methods

2.1. Cell lines, chemicals and reagents

The human mammary carcinoma cell line MDA-MB231 was from the American Type Culture Collection (Rockville, MD) and was cultured in Dulbecco's modified Eagle's medium (Biochrom, Basel, Switzerland) and 10% FCS (p/s, Life Technologies, Breda, the Netherlands). Dox was from Pharmacia B.V., Woerden, The Netherlands. Indomethacin was from Bufa B.V., Uitgeest, The Netherlands; Sc-236, NS-398 and celecoxib were from Pharmacia, Skokie, USA. PSC833 was from Novartis, Basel, Switzerland. Probenecid was from Sigma, St. Louis, MO, USA. Ko143 (fumitremorgan C analogue) was a kind gift from A. van Loevezijn, Laboratory of Organic Chemistry, University of Amsterdam (Amsterdam, The Netherlands). The fluorescent pump substrates, Syto16 (for Pgp), calcein acetoxymethylester (for MRP1) and Bodipy-prazosin (for BCRP), were from Molecular Probes, Eugene, OR, USA, and actionomycin D (7-AAD) was from Pharmingen, San Diego, CA, USA. For transfections, Fugene 6 transfection reagent was from Roche, Basel, Switzerland, and the NF-κB reporter construct NF-κB-luc was from Stratagene, Amsterdam, The Netherlands, and Renilla luciferase (pRL-SV40) was from Promega, Madison, WI, USA. The NF-κB inhibitor pyrrolidinedithiocarbamate (PDTC) was from Sigma, Zwijndrecht, The Netherlands.

2.2. Animal study

Female BALB/c nu/nu mice were from Iffa Credo (L'Arbresle, France) and were housed in individual ventilated cages under sterile conditions according to the Swiss guidelines for the care and use of laboratory animals. Sterile food and water were provided ad libitum. At start of the experiment, the mice were ≈10 weeks old. MDA-MB231 cells (2×10^6 cells/100 μl) were injected sc. in the left flank. After 2 weeks, animals containing a tumour with a volume (TV) of $100 \pm 20\ mm^3$ were selected and divided into four groups of eight mice each. TV was assessed by using a calliper measuring the two major diameters by the formula $TV = \pi/6\ (d1 \times d2)^{1/2}$. Mice (8 per experimental group, $n = 8$) were treated with vehicle (DMSO), celecoxib ($15\ mg\ kg^{-1}$), dox ($0.5\ mg\ kg^{-1}$) or a combination of celecoxib and dox. All agents were injected i.p. in a total volume of 125 μl. The mice were treated every other day for 30 days. TV and body weight were measured twice a week.

2.3. MTS assay

Cell proliferation was assessed by the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4- sulphophenyl)-2H-tetrazolium) tetrazolium assay (Cell Titer96 Aqueous, Promega). For this, 2×10^3 cells per well were seeded in triplicate in 90 μl culture medium in 96-well flat-bottom microculture plates and one day later the additives were added. After 4 d of culture, viable cells were determined by adding 20 μl MTS to each well and measuring OD 490 (Thermomax, Molecular Devices) after 2 h incubation. The results