BIOCHEMICAL PHARMACOLOGY 75 (2008) 427–437

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Interaction between celecoxib and docetaxel or cisplatin in human cell lines of ovarian cancer and colon cancer is independent of COX-2 expression levels

Marcel N.A. Bijman, Cristian A. Hermelink, Maria P.A. van Berkel, Adrie C. Laan, Maarten L. Janmaat, Godefridus J. Peters, Epie Boven *

Department of Medical Oncology, VU University medical center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 6 July 2007
Accepted 5 September 2007

Keywords:
Cyclooxygenase
Combination index
Cisplatin
Docetaxel
Celecoxib

ABSTRACT

Celecoxib, an inhibitor of cyclooxygenase-2 (COX-2), is being investigated for enhancement of chemotherapy efficacy in cancer clinical trials. We determined whether continuous exposure to celecoxib would increase the antiproliferative effects of a 1-h treatment with docetaxel or cisplatin in four human ovarian cancer cell lines. COX-2 protein could not be detected in these cell lines, because of which three COX-2 positive human colon cancer cell lines were included. Multiple drug effect analysis demonstrated additive to borderline antagonistic effects of celecoxib combined with docetaxel. Combination indices with values of 1.4–2.5 in all cancer cell lines indicated antagonism between celecoxib and cisplatin regardless whether celecoxib preceded cisplatin for 3 h, was added simultaneously or immediately after cisplatin. Apoptotic features measured in COX-2-negative H134 ovarian cancer cells and COX-2-positive WiDr colon cancer cells, such as the activation of caspase-3 and the number of cells in sub-G0 of the cell cycle, induced by docetaxel were increased in the presence of celecoxib, but were abrogated upon addition of celecoxib to cisplatin. Moreover, the G2/M accumulation in cisplatin-treated cells was less pronounced when celecoxib was present. Drugs did not affect p-Akt. Celecoxib upregulated p-ERK1/2 in H134 cells, but not in WiDr cells. Platinum–DNA adduct formation measured in WiDr cells, however, was reduced when celecoxib was combined with cisplatin. Taken together, our data demonstrate clear antagonistic effects when celecoxib is given concurrently with cisplatin, which is independent of COX-2 expression levels.

1. Introduction

The enzyme cyclooxygenase (COX) catalyzes prostaglandin synthesis from arachidonic acid. Two isoforms have been characterized of which COX-1 is a homeostasis protein constitutively expressed in a variety of tissues, while COX-2 expression is regulated by growth factors, cytokines and oncogenes. COX-2 overexpression can be found in both premalignant and malignant lesions. Induction of COX-2 has been shown to promote cell growth, inhibit apoptosis and enhance cell motility and adhesion [1,2]. Classical anticancer agents may upregulate COX-2 mRNA and protein levels in tumor cells, as has been described for cisplatin and taxanes [3–5].

Since COX-2 expression leads to a pro-survival effect, COX-2 inhibitors have been investigated for their potential to enhance chemotherapy efficacy. At first instance, non-steroidal anti-inflammatory drugs (NSAIDs) have been

* *Corresponding author*. Tel.: +31 20 4444336; fax: +31 20 4444079.
E-mail address: e.boven@vumc.nl (E. Boven).
0006-2952/$ – see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.bcp.2007.09.005

employed, but this class of agents also inhibits COX-1 function causing gastrointestinal injury. Thereafter, specific COX-2 inhibitors were developed to avoid side effects of NSAIDs related to COX-1 inhibition [6], such as celecoxib. Celecoxib specifically binds to COX-2, while little or no specific binding to COX-1 was observed [7]. Besides its anti-inflammatory activities, in relevant animal models of cancer celecoxib has been found to prevent colon tumor formation, to inhibit angiogenesis and to potentiate tumor response to radiation [8]. More specifically, celecoxib may enhance antitumor activity when combined with chemotherapy. As examples, celecoxib combined with docetaxel was more effective than docetaxel alone in the growth inhibition of A549 human lung tumors in nude mice [5]. The same combination potentiated apoptosis in human prostate cancer cells and had additive antitumor effects *in vivo* [9]. Combined with oxaliplatin it could enhance human colon cancer cell death *in vitro* [10]. Clinical trials have been conducted or are underway using celecoxib in combination with cytotoxic agents, such as gemcitabine and cisplatin in pancreatic cancer [11] and docetaxel in lung cancer [12]. Thus far, the benefit of celecoxib as an adjunct to chemotherapy regimens has not yet been established.

The most likely route of celecoxib to potentiate the efficacy of chemotherapy is considered via inhibition of COX-2. Recent experiments, however, provide evidence that celecoxib is also able to inhibit human cancer cell growth regardless of the presence of functional COX-2 [13–15]. It is, therefore, hypothesized that celecoxib improves chemotherapy outcome not only via COX-2 inhibition, but also by other, mostly unknown, mechanisms.

The functional activity of COX-2 and the effects of COX-2 inhibition have been mainly studied in colorectal cancer. COX-2 is overexpressed in approximately 90% of patients with colorectal adenocarcinomas [16]. A high level of COX-2 expression was correlated with more advanced stage and larger tumor size and might be related with reduced survival [17]. Various epidemiological studies have indicated that regular and prolonged intake of NSAIDs is associated with a 40–50% reduction in colorectal cancer incidence, most probably due to reduced prostaglandin synthesis as a consequence of decreased COX-2 activity [18]. Moreover, in experimental human colon cancer COX-2 inhibition potentiated the efficacy of cytotoxic agents, such as oxaliplatin, irinotecan and curcumin [10,19,20].

Although not frequently overexpressed, COX-2 can be detected in human ovarian tumors [21–23]. Expression of COX-2 was found to be associated with a significantly reduced median survival time [22] and levels were significantly higher in non-responding patients than patients responding to chemotherapy [23]. The well-known NSAID acetyl salicylic acid (aspirin) has been shown to inhibit OVCAR-3 human ovarian cancer cell growth *in vitro* [24]. Other studies in human ovarian cancer cell lines treated with the specific COX-2 inhibitor NS398, however, led to ambiguous effects; both induction of G0/G1 cell cycle arrest [25] and impairment of paclitaxel-induced apoptosis [26] have been reported. The exact role of COX-2 as a target for treatment in ovarian cancer remains to be elucidated.

In this study we investigated the possible synergism of celecoxib when combined with docetaxel or cisplatin in human ovarian cancer cell lines. Since these cell lines did not clearly express COX-2 protein levels, human colon cancer cell lines that contain COX-2 were included as control cell lines. We detected increased cytotoxic effects and apoptotic features when celecoxib was combined with docetaxel regardless of the expression levels of COX-2. Of interest, we consistently calculated antagonistic effects upon the combination of celecoxib with cisplatin, both in ovarian cancer as well as in colon cancer cells. We, therefore, investigated the mechanism of the antagonism between celecoxib and cisplatin. Evidence was found that celecoxib-treated cells were protected against the cisplatin-induced G2/M arrest as well as against platinum–DNA adduct formation in which p21 may play a role.

2. Materials and methods

2.1. Cell culture

Four human ovarian cancer cell lines: A2780, H134, OVCAR-3, IGROV-1 [27,28] and three human colon cancer cell lines: WiDr, HT29, SW1398 [29,30] were used for the experiments. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Breda, the Netherlands) supplemented with 10% fetal calf serum (FCS, Invitrogen), 100 U/ml penicillin and 100 μg/ml streptomycin (Bio-Whittaker, Verviers, Belgium) at 37 °C in 5% CO_2.

2.2. In vitro antiproliferative assay

The antiproliferative effects of docetaxel (kindly provided by Sanofi-Aventis, Antony, France), cisplatin (Bristol-Myers Squibb, Woerden, the Netherlands), celecoxib (kindly provided by Pfizer, Barceloneta, Puerto Rico) and combinations of docetaxel–celecoxib or cisplatin–celecoxib were measured in a 96-h antiproliferative assay. Cells were plated in quadruplicate in culture medium in 96-well plates at 3000 cells per well. After 24 h, cells were exposed to drug concentration ranges of docetaxel (1 h), cisplatin (1 h) or celecoxib (96 h). Docetaxel and cisplatin treatments were followed by a wash step and addition of fresh culture medium. Besides assessment of the antiproliferative effects of individual drugs, three different experimental designs were used: (1) a 3-h preincubation with celecoxib followed by a 1-h simultaneous exposure to docetaxel or cisplatin and a subsequent 96-h exposure to celecoxib, (2) a 1-h simultaneous exposure to docetaxel or cisplatin plus celecoxib and a subsequent 96-h exposure to celecoxib, (3) a 1-h exposure to docetaxel or cisplatin followed by a 96-h celecoxib exposure. Constant drug concentration ratios were applied in which docetaxel molar concentrations were 1000-fold lower than the celecoxib concentrations, while cisplatin and celecoxib concentrations were added in equal molar ratios.

The number of viable cells was determined by addition of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT; Sigma–Aldrich, Zwijndrecht, the Netherlands). The extinction of the formazan product was measured at 540 nm on a Multiscan plate reader (Thermo Biosciences, Breda, the Netherlands). Results were expressed in IC50