Docetaxel in ovarian cancer

Bijman, M.N.A.

2009

document version
Publisher's PDF, also known as Version of record

Summary

Standard treatment of ovarian cancer consists of cytoreductive surgery followed by chemotherapy consisting of a combination of platinum compounds and taxanes. To date, the combination of carboplatin and paclitaxel is the chemotherapy of choice. In a recent study in patients with advanced ovarian cancer, it has been demonstrated that a combination of carboplatin and docetaxel results in a similar period of progression-free survival with a superior benefit of the quality of life as compared to treatment with carboplatin and paclitaxel. In the laboratory, docetaxel has shown some advantages over paclitaxel. Both drugs have the same mechanism of action in dividing cells, i.e. stabilization of microtubules because of which depolymerization is prevented and mitosis cannot be completed. There are, however, subtle differences between these drugs. Docetaxel has approximately a 2-fold higher affinity for ß-tubulin binding and a longer intracellular retention time. Docetaxel is more potent in the stabilization of microtubules than paclitaxel. In addition to antitumor activity, taxanes are also known to inhibit angiogenesis, a process required for tumor growth and the formation of metastases.

In this thesis, several aspects of docetaxel were investigated which might be useful to improve the treatment of cancer. Human ovarian cancer cell lines and human umbilical vein endothelial cells (HUVECs) were used as *in vitro* test systems. First, the potential of docetaxel to inhibit endothelial cell motility, a process required for new vessel formation, was explored. The drug was also investigated for possible interference with tumor cell motility, because primary ovarian cancer is known for seeding tumor cells in the peritoneal lining of the abdominal cavity. Since cyclooygenase-2 (COX-2) may be an important enzyme associated with tumor cell survival, it was then investigated whether a combination of docetaxel with the COX-2 inhibitor, celecoxib, would improve antitumor effects. Last, since ovarian cancer cells express members of the Human Epidermal growth factor Receptor (HER) family, HER-targeting monoclonal antibodies were explored whether antiproliferative effects would be increased by their addition to docetaxel.

In **Chapter 2** several conventional cytotoxic agents were investigated for possible antiangiogenic properties by the use of HUVECs. To that end, a series of microtubule-targeting agents were selected, since these compounds have been reported to inhibit cell motility by affecting the dynamics of microtubules. These drugs were docetaxel, known to stabilize microtubules whereby the dynamic reorganization and depolymerization are inhibited; epothilone B, which are not related to taxanes but are also able to stabilize microtubules, and vinblastine, known to inhibit both tubulin polymerization and mitotic spindle formation. Two other well-known anticancer agents were included in the experiments, being cisplatin and doxorubicin and both known to affect DNA integrity. First, the antiproliferative effects of the cytotoxic agents were investigated in HUVECs, which were exposed to the drugs for a period of 1 h, in order to determine the concentration of each drug that inhibited a maximum of 10% of cell growth as compared to control cell growth. This concentration was defined as the Highest Non-Toxic Concentration (HNTC).

Thereafter, the HNTC was used in all further tests and was routinely checked in a parallel antiproliferative assay. Endothelial cell motility was assessed in the wound assay for migration, in a transwell system for invasion and after seeding on top of extracellular matrix gel for organization ('tube formation'). Thus, in these assays equitoxic concentrations of the drugs were tested. Cisplatin and doxorubicin did not inhibit endothelial cell motility. In contrast, all microtubule-targeting agents significantly inhibited angiogenic features of HUVECs (p <0.05). The inhibition of migration and invasion appeared to be most pronounced for docetaxel, although this was not significantly different from that obtained with epothilone B and vinblastine. In the next experiment structural changes in endothelial cell morphology were examined in the wound assay after a 1-h drug exposure at HNTC by visualization of tubulin and F-action by fluorescence microscopy at 8 h after wounding. Control cells and cells treated with cisplatin or doxorubicin showed the same morphology; actin stress fibers could be seen in the cell body and cellular actin protrusions were filled with tubulin in the direction of movement. HUVECs treated with microtubule-targeting agents, however, seemed to reside at the edge of the wound. Both docetaxel and epothilone B led to a significantly higher signal intensity staining of tubulin (p <0.01), but tubulin intensity was not changed in vinblastine-treated cells as compared to that in control cells. HUVECs treated with microtubule-targeting agents contained less stress fibers. Instead, rings of F-actin had formed around the nucleus, suggesting loss of correct cell polarization. Actin protrusions in the direction of movement were virtually absent. In the last experiment the activities of two members of Rho GTPases, a family of molecules that tightly coordinate motility, were examined. Cdc42 is known to be a regulator of polarity of the cell enabling motility to be initiated in the desired direction. Rac1 can induce the formation of extensions (lamellipodia) and stimulates actin polymerization at the leading edge of the cell, together with the formation of new adhesion sites to the matrix. The activity of both Rac1 and Cdc42 are tightly regulated by actin and tubulin dynamics. HUVECs were treated with HNTC of the drugs for 1 h. Thereafter, total and active protein levels were measured and were compared to baseline levels. As expected, cisplatin and doxorubicine did not affect the activities of Rac1 and Cdc42. In contrast, microtubule-targeting agents could efficiently inhibit the activities of both proteins. Altogether, these experiments in HUVECs showed that microtubule-targeting agents, such as docetaxel, can inhibit endothelial cell motility which is associated with inhibition of Rac1 and Cdc42 and changes in the endothelial cytoskeleton. Therefore, apart from their direct antitumor effects these drugs may be useful for inhibition of angiogenesis in malignancies.

In **Chapter 3** the microtubule-targeting agents docetaxel, epothilone B and vinblastine were analyzed whether these drugs could inhibit ovarian cancer cell motility. In the experiments, cisplatin and doxorubicin were included as well as cytochalasin D, a compound known to inhibit F-actin polymerization. Cytochalasin D is known to reduce actin filament mass and stabilizes the barbed end dynamics of the filaments. Human ovarian cancer cell lines A2780, H134, OVCAR-3, IGROV-1 and SKOV-3 were selected. The HNTC of each drug was calculated in each cell line after a 1-h drug exposure time. In addition, drug concentrations were calculated which inhibited 50% of cell

growth (IC50) when compared to control cell growth. Ovarian cancer cell motility was investigated in a wound assay for migration and in a transwell system for invasion. Spontaneous migration and invasion was grossly absent in the case of A2780 and H134 cells. Migration and invasion in IGROV-1, OVCAR-3 and SKOV-3 were not affected by cisplatin and doxorubicin. Microtubule-targeting agents only slightly inhibited these processes. Cytochalasin D was significantly effective at HNTC in the migration assay (p <0.001) and at IC50 in the invasion assay (p <0.01). The cytoskeletal changes in IGROV-1, OVCAR-3 and SKOV-3 were visualized in the wound assay. Cells were exposed for 1 h to docetaxel or cytochalasin D at IC50 and wounded and were stained for tubulin and F-action 8 h thereafter. Migrating control cells showed broad lamellipodia filled with microtubules in the direction of movement. Upon treatment with docetaxel, only a subtle increase in the tubulin signal was noticed. Upon cytochalasin D exposure, however, patchy layers of F-action were visible around the cells and characteristic broad lamellipodia were lost. In OVCAR-3 cells it was then determined whether docetaxel or cytochalasin D might result in a reduced functionality of Rac1 and Cdc42. Total protein levels were not affected. Rac1 activity was slightly inhibited by docetaxel, which was more pronounced in the case of cytochalasin D treatment. Active Cdc42, however, was unaltered. Altogether, at HNTC or even concentrations inhibiting 50% of ovarian cancer cell growth, microtubule-targeting agents only marginally inhibited cell motility. Of interest, the actin-targeting agent cytochalasin D was most potent in hampering both cell migration and invasion. Therefore, agents that target the actin cytoskeleton may be attractive for drug development. Such compounds might specifically be useful in patients with ovarian cancer after debulking surgery to prevent the intra-abdominal metastatic process.

The enzyme COX catalyses 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. Induction of COX-2 has been shown to promote cell growth, inhibit apoptosis and enhance cell motility and adhesion. COX-2 inhibitors have been and are under investigation for their potential to enhance chemotherapy efficacy. In **Chapter 4** it was examined whether the COX-2 inhibitor, celecoxib, would increase the antitumor effects of docetaxel or cisplatin in human ovarian cancer cell lines A2780, H134, OVCAR-3 and IGROV-1. Since COX-2 could not be detected in these cell lines, COX-2-positive human colon cancer cell lines WiDr, HT29 and SW1398 were included in the experiments. In antiproliferative assays, the effects of celecoxib, docetaxel and cisplatin and the combination of celecoxib with each of the anticancer agents were analyzed, while possible antagonistic, synergistic or additive effects were calculated with the Chou and Talalay formula. Cell exposure to celecoxib was continuous, while a 1-h exposure time was used for docetaxel and cisplatin. It was clearly shown that the combination of celecoxib and cisplatin induced antagonistic antiproliferative effects, while celecoxib plus docetaxel produced additive or slightly antagonistic antiproliferative effects. The antagonistic effects of celecoxib when combined with cisplatin were calculated regardless whether celecoxib preceded the drugs for a period of 3 h, was added simultaneously, or was added after the 1-h exposure of cisplatin. Also, these antagonistic effects were calculated in all

cell lines regardless of COX-2 expression levels. In further experiments, drug concentrations were used inducing 50% cell growth inhibition when combined, while celecoxib preceded docetaxel or cisplatin with 3 h. By analysis of caspase-3 activity and calculation of the sub-G0 fraction of the cell population by fluorescence-activated cell sorting (FACS) in COX-2-negative H134 cells and COX-2-positive WiDr cells, it was demonstrated that celecoxib increased the apoptotic features from docetaxel, but inhibited cisplatin-induced apoptosis. Cell cycle analysis showed that exposure to celecoxib, docetaxel or the combination did not change the distribution in H134 and WiDr cells. In contrast, a G2/M accumulation by cisplatin in both cell lines was less pronounced upon addition of celecoxib. The decrease in the G2/M fraction was even significant in WiDr cells (p <0.05). In order to understand the antagonism between celecoxib and cisplatin, p-Akt and p-ERK1/2 were investigated upon treatment of H134 and WiDr cells. p-Akt levels were not affected. p-ERK1/2 levels increased in H134, but not in WiDr, and could, therefore, not explain the negative drug interaction. In a last experiment in WiDr cells platinum-DNA adducts were measured by atomic absorption spectrometry to calculate cisplatin-induced DNA damage. Of interest, at the end of the exposure to cisplatin it appeared that the platinum-DNA adduct concentration was approximately 2-fold lower in the presence of celecoxib. Also, the decrease in platinum-DNA adducts after withdrawal of cisplatin was more pronounced in the presence of celecoxib. Altogether, the experiments showed that tumor cells do not need to express COX-2 for celecoxib to be effective. In general, celecoxib combined with docetaxel resulted in additive effects and increased apoptotic features. For the combination of celecoxib and cisplatin, however, antagonistic effects were measured regardless of COX-2 expression levels. Therefore, clinical trials combining celecoxib with cisplatin should be carried out with caution.

Currently, anticancer drugs are being combined with targeting agents to improve the treatment of cancer. In this respect, members of the HER family are attractive targets. In ovarian cancer, EGFR expression can be found in 29-62% of samples, while approximately 13-35% will express HER2 to some degree. The prognostic value of these receptors for survival is not clear. Only recently, HER3 has gained interest in ovarian cancer. Positive HER3 expression was observed in 53.4% of ovarian cancer patients and was associated with decreased survival. In **Chapter 5** it was investigated whether inhibition of HER family members would increase the antitumor effects of docetaxel. EGFR, HER2, HER3, HER4 expression levels differed among A2780, OVCAR-3, IGROV-1, H134 and SKOV3 ovarian cancer cell lines. For the experiments OVCAR-3 (high EGFR, low HER2, presence of HER3), IGROV-1 (low EGFR, moderate HER2, presence of HER3) and SKOV-3 (high EGFR, high HER2, absence of HER3) were selected because of differential expression levels of receptors. Docetaxel concentrations inhibiting 50% of cell growth after an exposure time of 1 h were used in the experiments. For inhibition of receptor function monoclonal antibodies cetuximab (against EGFR), trastuzumab (against HER2) and pertuzumab (against the region in HER2 required for dimerization with HER3; blocks also EGF-mediated phosphorylation of HER2) were selected. Monoclonal antibodies were added 2 h prior to the 1-h exposure to docetaxel and were present throughout the experiments. The antiproliferative assay revealed that cetuximab alone or added to

docetaxel significantly reduced cell growth in OVCAR-3 and IGROV-1 (p <0.05). HER2-targeting antibodies alone were not effective. Co-addition of cetuximab and pertuzumab to docetaxel in OVCAR-3 and IGROV-1, and, to a lesser extent trastuzumab added to docetaxel in OVCAR-3, reduced cell growth inhibition from docetaxel further. SKOV-3 cell growth was not significantly affected by any of the antibodies. The caspase-3 activity assay was used as a measure of apoptosis. Cetuximab added to docetaxel increased caspase-3 activity in OVCAR-3, which was also the case when cetuximab and pertuzumab were both added to docetaxel in IGROV-1 and SKOV-3 (p <0.05). It was then examined whether receptors could be activated in the three cell lines by exposure to Epidermal Growth Factor (EGF) or heregulin (HRG). EGF could induce p-EGFR which was completely inhibited by cetuximab in OVCAR-3 and IGROV-1, but partially in SKOV-3. Cetuximab also inhibited p-ERK1/2 in all cell lines. Increased p-Akt upon stimulation by EGF in OVCAR-3 was abrogated by cetuximab. HRG stimulation induced p-EGFR in OVCAR-3, which was again abrogated by cetuximab. Induction of p-ERK1/2 and p-Akt in OVCAR-3, however, was only abrogated by cetuximab in combination with pertuzumab. In HRG-stimulated IGROV-1 and SKOV-3 cells, p-EGFR was not increased. Pertuzumab, however, could inhibit p-ERK1/2 in IGROV-1 cells when added to cetuximab. Thus, functional EGFR- and HER3-signaling routes could be demonstrated in OVCAR-3 and IGROV-1, but hardly in SKOV-3. In a last experiment it was investigated, whether EGF and HRG could stimulate HER2 by analysis of tyrosine phosphorylation as a measure of HER2 activation. Both in OVCAR-3 and IGROV-1 phosphorylation of HER2 could be detected, which was efficiently abrogated by pertuzumab. In SKOV-3 baseline phosphorylation of HER2 was already present, which was neither increased by EGF or HRG, nor abrogated by pertuzumab. Thus, a functional HER2 signaling route could be detected in OVCAR-3 and IGROV-1, but not in SKOV-3. Altogether, the experiments carried out in the ovarian cancer cell lines with different expression levels of HER family members illustrated that inhibition of functional receptors may increase the antitumor effects of docetaxel.

The general conclusion of this thesis is, that docetaxel shows anti-angiogenic properties in a concentration that does not affect endothelial cell proliferation. Docetaxel, however, is not clearly effective at subtoxic concentrations in the inhibition of ovarian cancer cell motility. In general, when docetaxel is combined with celecoxib this will result in additive antiproliferative effects in ovarian cancer cells, although the combination promotes apoptosis. In ovarian cancer cells expressing HER family members, inhibition of functional receptors can increase sensitivity to docetaxel. This thesis has given insight in several aspects of docetaxel, which may be of help to better understand its mechanism of action and to select combinations with targeting agents to improve the treatment of ovarian cancer.