Combination therapy of zibotentan with cisplatinum and paclitaxel is an effective regimen for epithelial ovarian cancer[1]

Laura Rosanò, Roberta Cianfrocca, Francesca Spinella, Valeriana Di Castro, Pier Giorgio Natali, and Anna Bagnato

Abstract: In human ovarian carcinoma, the endothelin-1 (ET-1)/endothelin A receptor (ET_AR) axis is overexpressed, correlating with tumor grade. Moreover, ET_AR activation by ET-1 affects cell proliferation, survival, angiogenesis, and invasion. ET_AR blockade with zibotentan (ZD4054), a specific ET_AR antagonist, significantly inhibits ovarian cancer growth in vitro and in vivo, underscoring the relevance of this pathway as a target for cancer therapy. Since clinical trial results have defined the combination of platinum and taxane as the standard of care in the management of ovarian cancer, here we explored the therapeutic efficacy of the integration of zibotentan with cytotoxic drugs having different modes of action. We found that the combination of zibotentan with cisplatinum as well as zibotentan with paclitaxel was more effective at inhibiting ovarian cancer HEY cell proliferation induced by endogenous ET-1 than were the single agents alone. However, a significantly enhanced efficacy was observed when we combined zibotentan, cisplatinum, and paclitaxel. Accordingly, in HEY xenografts the coadministration of zibotentan with cisplatinum enhanced the efficacy of the cytotoxic drug alone in controlling tumor growth, associated with reduction in proliferation index and microvessel density. Remarkably, the combination of zibotentan with both cisplatinum and paclitaxel was very effective in inhibiting tumor growth, neovascularization, and cell proliferation, representing a preclinical endpoint to guide combination therapy in clinical trials.

Key words: ovarian cancer, ET-1, ET_A receptor, zibotentan, combination therapy.

Résumé : Dans le carcinome ovarien humain, l'axe endothéline-1 (ET-1)/récepteur A de l'ET (RET_A) est surexprimé, en relation avec le grade tumoral. De plus, l'activation du RET_A par l'ET-1 entraîne de nombreux effets cellulaires, y compris la prolifération, la survie, l'angiogenèse et l'invasion. Le blocage du RET_A au moyen du zibotentan (ZD4054), antagoniste spécifique de RET_A, inhibe significativement la croissance du cancer ovarien in vitro et in vivo, soulignant l'intérêt de cette voie comme cible dans le traitement du cancer. Les résultats d'essais cliniques ayant défini l'association platine-taxane comme une norme de soins dans le traitement du cancer ovarien, nous avons examiné l'efficacité thérapeutique de l'association de zibotentan et de médicaments cytotoxiques ayant différents modes d'action. Nous avons constaté que l'association zibotentan–cisplatine et zibotentan–paclitaxel inhibe plus efficacement la prolifération des cellules HEY de carcinome ovarien induite par l'ET-1 endogène que l'emploi de médicaments seuls. Toutefois, l'efficacité a augmenté de manière significative lorsque nous avons combiné zibotentan, cisplatine et paclitaxel. Ainsi, dans les xénogreffes de cellules HEY, la coadministration de zibotentan et de cisplatine a stimulé l'efficacité du médicament cytotoxique pour contrôler la croissance de la tumeur, comme indiqué par la diminution de l'index de prolifération et de la densité des microvaisseaux. L'association zibotentan–cisplatine–paclitaxel a été très efficace pour inhiber la croissance tumorale, la néovascularisation et la prolifération cellulaire, et représente un critère préclinique pour guider la polythérapie dans les essais cliniques.

Mots-clés : cancer ovarien, ET-1, récepteur ET_A, zibotentan, polythérapie.

[Traduit par la Rédaction]

Introduction

Ovarian cancer has a distinctive biology and behavior at the clinical, cellular, and molecular levels (Bast et al. 2009). Despite ongoing efforts to develop an effective screening strategy, only 20% of ovarian cancers are diagnosed at an early stage of tumor progression and can be cured using currently available therapy. After the disease has metastasized, the cure rate decreases substantially, and although patients respond to a combination of platinum- and taxane-based chemotherapy, small numbers of drug-resistant cells can persist and remain dormant in the peritoneal cavity, to grow progressively, leading to the death of the patient despite aggressive treatment of recurrent disease (Hennessy et

Received 23 October 2009. Accepted 22 March 2010. Published on the NRC Research Press Web site at cjpp.nrc.ca on 22 June 2010.

L. Rosanò, R. Cianfrocca, F. Spinella, V. Di Castro, P.G. Natali, and A. Bagnato.[2] Laboratory of Molecular Pathology, Regina Elena National Cancer Institute, Via Delle Messi D'Oro 156, Rome, Italy.

[1]This article is one of a selection of papers published in the two-part special issue entitled 20 Years of Endothelin Research.
[2]Corresponding author (e-mail: bagnato@ifo.it).

al. 2009). Given the heterogeneity of this disease at the cellular and molecular levels, and the large number of molecules and signaling pathways that are most frequently activated or overexpressed in ovarian cancers, an immediate challenge is to identify pathways for which inhibition would provide synthetic lethality using conventional drugs together with targeted therapies (Bast et al. 2009). Among new targets, great emphasis is placed on targeting the endothelin (ET) axis, which is capable of controlling the interactions of cancer cells with stromal components in the tumor (Bagnato et al. 2008). The ET family comprises three 21-amino acid peptides, ET-1, ET-2, and ET-3, which are potent vasoconstricting peptides involved in the pathophysiology of different malignancies (Levin 1995; Masaki 2000). ETs mediate their action by activating two G protein-coupled receptor (GPCR) subtypes, ET_A and ET_B. The ET_BR is expressed in endothelial cells, but ET_AR expression here is almost undetectable (Morbidelli et al. 1995; Salani et al. 2000*a*). In these cells, ET-1 acts as an angiogenic factor directly modulating various stages of neovascularization, including endothelial cell proliferation, migration, invasion, protease production, and tube formation, through ET_BR. Moreover, ET-1 can act indirectly to induce the release of major angiogenic factors, such as vascular endothelial growth factor (VEGF), from tumor cells expressing ET_AR (Salani et al. 2000*a*). ET-1 also contributes to endothelial cell integrity by acting as a survival factor (Shichiri et al. 1997). In epithelial ovarian cancer, the levels of ET-1 are markedly elevated in the ascites of patients, and ET-1 and ET_AR are overexpressed and activated in 85% of ovarian tumors, correlating with advanced stage of disease (Rosanò et al. 2005), expression of VEGF, and tumor-induced vascularization (Salani et al. 2000*b*). Upon being activated, the ET_AR in ovarian cancer cells mediates activation of different signaling pathways, including mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)-dependent Akt activation, epidermal growth factor receptor (EGFR) transactivation, p125 focal adhesion kinase and paxillin activation, involved in the control of cell proliferation, survival, VEGF-mediated angiogenesis, migration, invasion, and metastatic spread (Bagnato et al. 2005). In particular, it has been demonstrated in a variety of cell types that ET-1 is an antiapoptotic factor, as demonstrated by the inhibition of chemotherapy drug-induced apoptosis, that modulates cell survival pathways through a Bcl-2-dependent and PI3K-mediated Akt activation (Del Bufalo et al. 2002; Nelson et al. 2005). Specific ET_AR antagonism may therefore provide an additional approach to the treatment of carcinoma, whereby ET_AR blockade could result in tumor growth inhibition by reducing tumor growth as well as by inducing apoptosis. Emerging experimental and preclinical data demonstrate that interfering with ET_AR pathways provides an opportunity for the development of new mechanism-based antitumor strategies by using an ET_AR antagonist alone and in combination with cytotoxic drugs or molecular inhibitors (Akhavan et al. 2006; Bagnato and Rosanò 2008; Banerjee et al. 2007).

Among various ET_AR antagonists, zibotentan is an orally bioavailable ET_AR antagonist that potently and specifically binds to the ET_AR, blocking signal transduction pathways implicated in cancer cell proliferation and other host-dependent processes promoting cancer growth (Growcott 2009; Morris et al. 2005; Rosanò et al. 2006, 2007*a*). Preclinical data showed that treatment with zibotentan produced tumor growth inhibition in well-established HEY ovarian carcinoma xenografts that was comparable with that achieved by paclitaxel. More marked and prolonged tumor growth inhibition was obtained by combined treatment of zibotentan with paclitaxel, associated with marked reduction of various tumor progression markers and tumor neovascularization and increased apoptosis (Rosanò et al. 2007*b*).

Currently, the standard first-line chemotherapy regimen in ovarian cancer is systemic administration of a platinum-based chemotherapy (cisplatinum or carboplatin) combined with a taxane (paclitaxel or docetaxel). This therapy elicits a significant response rate, with most patients with ovarian cancer able to achieve a period of remission at the culmination of first-line therapy (Hennessy et al. 2009). Because of the high levels of recurrence associated with this treatment option, in this study we extended the preclinical evaluation of zibotentan by in vitro and in vivo testing the efficacy of zibotentan plus cisplatinum and paclitaxel to improve the therapeutic outcome of this chemotherapeutic regimen.

Materials and methods

Materials

Clinical grade zibotentan (ZD4054) was kindly provided by AstraZeneca (Macclesfield, UK). Paclitaxel was kindly provided by Bristol-Myers (Sermoneta, Italy), and cisplatinum by Teva Pharmaceuticals (Utrecht, Netherlands).

Cells and cell culture conditions

The human ovarian carcinoma cell line HEY, generously provided by Prof. Giovanni Scambia (Catholic University School of Medicine, Rome, Italy) and previously characterized for ET-1 receptor expression and for ET-1 production (Bagnato et al. 1995, 1999), were cultured in RPMI containing 10% fetal calf serum (FCS) and 1% penicillin–streptomycin at 37 °C under 5% CO_2 and 95% air. The cells were serum-starved by incubation for 24 h in serum-free RPMI. All culture reagents were from Invitrogen (Paisley, UK).

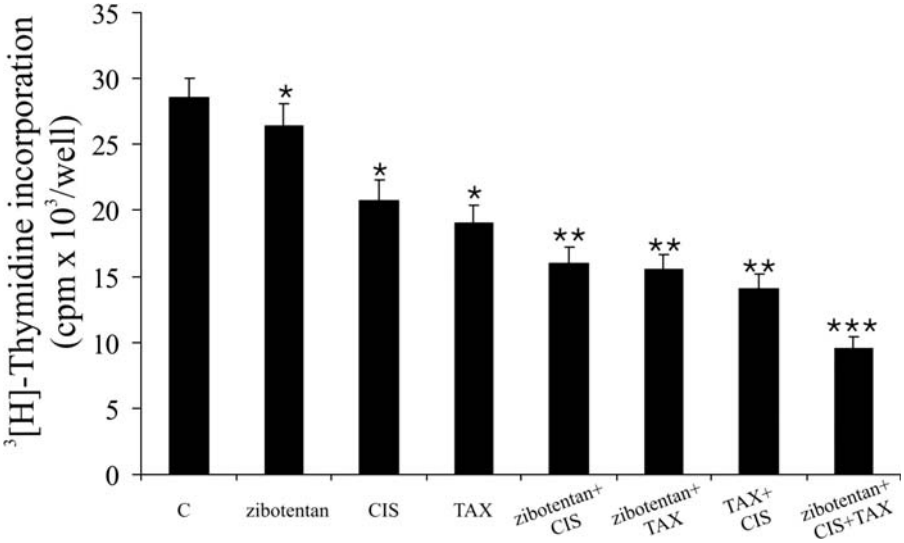
Thymidine incorporation assay

Cells were seeded in 96-well plates at approximately 80% confluence (2×10^6 cells/well) and incubated in serum-free medium for 24 h to induce quiescence before adding 100 nmol/L ET-1. After 24 h, when cells were confluent, 1 μCi of [methyl-^{3}H]thymidine (5.0 Ci/mmol) was added to each well (1 MBq = 27.03 μCi). Six hours later, culture media were removed, and cells were washed 3 times with PBS, treated with 10% trichloroacetic for 15 min, washed twice with 100% ethanol, and solubilized in 0.4 mol/L sodium hydroxide. The cell-associated radioactivity was then determined by liquid scintillation counting. Responses to all treatments were assayed in 6 replicates, and results were expressed as the means of 3 separate experiments.

Xenografts in nude mice

Female athymic nude (*nu*/*nu*) mice, 4–6 week of age (Charles River Laboratories, Milan, Italy), were treated fol-

Fig. 1. Effect of zibotentan in combination therapy with paclitaxel and cisplatinum on ovarian carcinoma cell proliferation. Serum-starved HEY cells were treated with zibotentan (1 μmol/L), paclitaxel (TAX, 60 nmol/L), or cisplatinum (CIS, 1 μmol/L), alone or in combination, before measuring [^{3}H]thymidine incorporation. Combination therapies evaluated were zibotentan + CIS, zibotentan + TAX, TAX + CIS, or zibotentan + CIS + TAX. Data are means ± SD of 6 duplicate determinations of 3 separate experiments. *, Significant at $p < 0.001$ compared with control (C); **, $p < 0.005$ compared with single treatments; ***, $p < 0.001$ compared with zibotentan + CIS or zibotentan + TAX.



lowing the guidelines for animal experimentation of the Italian Ministry of Health. Mice were injected s.c. into one flank with 1.5×10^6 viable HEY cells. After 7 days, when tumors reached approximately 0.2–0.3 cm in diameter, mice were randomized into groups ($n = 10$) to receive one of the following treatments for 21 days: zibotentan (diluted in PBS) at a daily dose of 10 mg/kg, paclitaxel 20 mg/kg per dose given i.v. 3 times a day every 4 days, cisplatinum 5 mg/kg i.p. one time on day 1, zibotentan and cisplatinum, zibotentan and paclitaxel, or zibotentan plus both cisplatinum and paclitaxel. Control mice were injected with the drug vehicle. Tumor size was measured with calipers and was calculated using the formula $\pi/6 \times$ larger diameter $\times$ (smaller diameter)^2.

Immunohistochemical analysis

Indirect immunoperoxidase stain of tumor xenografts was performed on acetone-fixed 4 μm tissue sections, as described previously (Rosanò et al. 2007*a*). Briefly, sections were incubated with monoclonal rat anti-mouse CD31 (PECAM-1 (platelet–endothelial cell adhesion molecule 1); generously donated by Dr. A. Mantovani, Mario Negri Institute, Milan, Italy), and with anti-Ki67 monoclonal antibody (clone MIB1; Ylem, Rome, Italy). The avidin–biotin assays were performed using the Vectastain Elite kit (for non-murine primary antibodies) and Vector MOM immunodetection kit (for murine primary antibodies) obtained from Vector Laboratories (Burlingame, USA). Mayer's hematoxylin was used as a nuclear counterstain. Negative control stain was represented by sections in which the incubation with the primary antibody was either omitted or substituted by isotype-matched immunoglobulins. The evaluation of microvessel density was performed by 2 independent observers on a $\times 200$ magnification field according to the criteria of Weidner et al. 1992. Cells positive for Ki67 were expressed as the percentage of tumor cells with nuclear staining counted in 5 separate $\times 40$ microscopic fields (at least 200 cells per field were counted).

Statistical analysis

Statistical analyses used Student's *t* test for in vitro studies. The time course of tumor growth was compared across the groups by two-way ANOVA, with group and time as variables. All statistical tests were two-sided. A probability value of $p < 0.05$ was considered statistically significant. All statistical analyses were performed using commercial software (SPSS, Chicago, USA).

Results

Combination of zibotentan and chemotherapeutic drugs inhibits cell proliferation

The potential antiproliferative effect of zibotentan in combination with paclitaxel and cisplatinum was evaluated on HEY cells that release ET-1 and express functional ET_AR (Bagnato et al. 1995, 1999; Rosanò et al. 2005). Serum-starved cell lines were exposed to zibotentan (1 μmol/L), paclitaxel (60 nmol/L), or cisplatinum (1 μmol/L), alone or in combination. As measured by the [^{3}H]thymidine incorporation assay, a significant inhibition of cell proliferation induced by endogenous ET-1 was observed in zibotentan-treated cells (Fig. 1). An additional reduction in proliferation was observed after treatment with combinations of either zibotentan and cisplatinum or zibotentan and paclitaxel compared with the inhibition produced by single agents (Fig. 1). However, the combination of zibotentan with both cisplatinum and paclitaxel was more effective than the inhibition produced by double treatments, suggesting that the specific blockade of the ET_AR with zibotentan cooperates with conventional cytotoxic drugs to potentiate the effect of paclitaxel and cisplatinum by inhibiting cell proliferation.

Fig. 2. Antitumor activity of zibotentan with paclitaxel and cisplatinum on established HEY human ovarian carcinoma xenografts. Mice were given injections of 1.5×10^6 HEY cells s.c. into the dorsal flank. After 7 days, randomized mice were treated for 21 days with vehicle (control), cisplatinum (CIS), paclitaxel (TAX), zibotentan, zibotentan + CIS, zibotentan + TAX, or zibotentan + CIS + TAX. Values are means ± SD of 3 different experiments, using a total of 70 mice for each experiment. The comparison of the time course of tumor growth curves by two-way ANOVA with group-by-time interaction for tumor growth was statistically significant ($p < 0.005$).

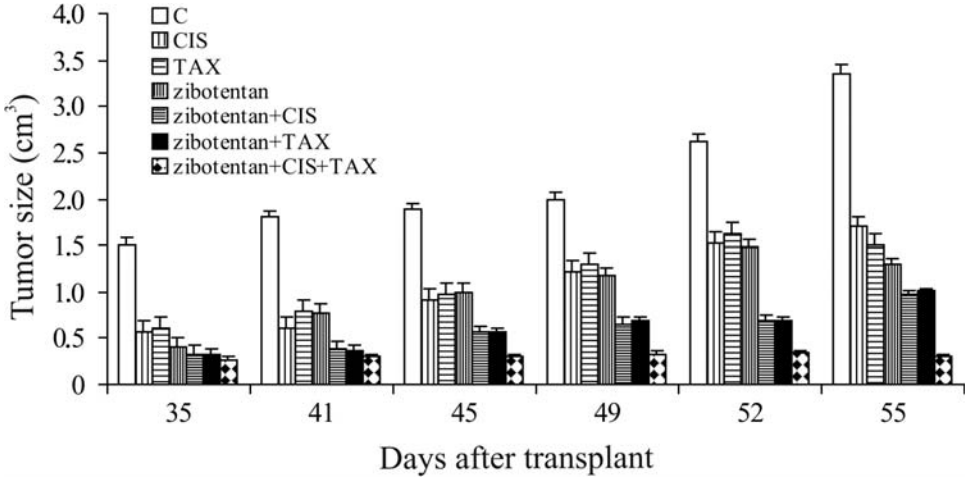


Table 1. Immunohistochemical analysis of HEY xenografts after treatment with zibotentan in combination with cytotoxic drugs.

Treatment	Tumor volume, cm^3	Median vessel density	Proliferation index Ki67
Control	3.36±0.09	68±5	38.1±1.9
Zibotentan	1.30±0.69*	26±2*	23.2±2.3*
TAX	1.50±0.12*	32±2*	26.6±2.0*
CIS	1.70±0.11*	25±2*	27.9±2.3*
Zibotentan + TAX	1.02±0.02†	19±2†	15.5±2.6†
Zibotentan + CIS	0.98±0.04†	18±2†	14.9±2.1†
Zibotentan + CIS + TAX	0.30±0.02†	11±1†	9.7±1.4†

Note: Mice were treated for 21 days with zibotentan, paclitaxel (TAX), cisplatinum (CIS), zibotentan + CIS, zibotentan + TAX, or zibotentan + CIS + TAX. Immunohistochemical analysis was performed 55 days after tumor injection. Vessel counts were assessed by light microscopy after staining for CD31. Areas containing the highest numbers of capillaries and small venules were identified by scanning at low power, and individual vessel counts were performed at a magnification of × 200. The relative number of proliferating cells was quantitatively assessed in 5 randomly selected fields at a magnification of × 40. Values are means ± SD, and each group consisted of 10 mice. *, Significant at $p < 0.05$ compared with control; †, $p < 0.002$ compared with single treatments.

Combination of zibotentan and chemotherapy inhibits growth of human HEY ovarian carcinoma in nude mice

The potential antitumor effect of combination treatment of zibotentan with cisplatinum plus paclitaxel in vivo was assessed in murine tumor xenografts. Human HEY cells were grown as subcutaneous tumors in nude mice. After 7 days of tumour growth, when established xenografts were palpable, mice were treated with single treatments of zibotentan, cisplatinum, or paclitaxel or combination treatments of zibotentan with cisplatinum, zibotentan with paclitaxel, or zibotentan with both cisplatinum and paclitaxel. Treatment with zibotentan produced a 61% inhibition of tumor growth on day 55 after tumor injection ($p < 0.001$ compared with control; Fig. 2); this effect was comparable with that achieved with either paclitaxel or cisplatinum. We next evaluated whether the cooperative effects observed for zibotentan with paclitaxel or cisplatinum in vitro were also present in vivo. A further significant growth inhibitory effect was seen when zibotentan was used in combination with cisplatinum (70%) or in combination with paclitaxel (69%). However, as observed in vitro, a more marked and sustained growth inhibitory effect was found when we combined zibotentan with both chemotherapeutic agents (90%). The combination treatment at the dose and schedule tested was well tolerated, as indicated by the absence of weight loss or other signs of acute or delayed toxicity. To demonstrate that the inhibition of tumor growth observed in treated mice was accompanied by a significant reduction in tumor cell proliferation and vascularization, tissue sections of HEY tumors on day 55 after tumor cell injection were analyzed by immunohistochemistry (Table 1). Tumor-induced vascularization, quantified by staining with anti-CD31, was significantly reduced compared with controls after treatment with zibotentan, paclitaxel, or cisplatinum alone (61%, 53%, and 57%, respectively). In mice treated with either zibotentan plus paclitaxel or zibotentan plus cisplatinum, this inhibition

reached approximately 70% ($p < 0.002$ compared with single agents); however, a more marked inhibitory effect was observed when zibotentan was combined with both cisplatinum and paclitaxel (80%). In parallel, we demonstrated that the number of proliferating tumor cells, measured by the number of Ki67-positive cells, was reduced approximately 38%, 30%, and 31% in tumors of mice treated with zibotentan, paclitaxel, or cisplatinum as single agents, respectively, compared with control mice ($p < 0.05$). This inhibition of proliferation was approximately 57% in mice treated with combinations of either zibotentan plus paclitaxel or zibotentan plus cisplatinum. As was shown for tumor vascularization, the combination of zibotentan with both cisplatinum and paclitaxel similarly resulted in approximately 75% reduction in the number of Ki67-positive cells ($p < 0.002$ compared with single agents). Collectively these findings confirm the potential cooperation of zibotentan with cisplatinum and paclitaxel to enhance the therapeutic efficacy of cytotoxic drugs.

Discussion

Despite many advances in the field of cancer therapeutics, ovarian cancer continues to be a leading cause of death, in part due to the failure of current chemotherapy regimens (Bast et al. 2009). Drug development strategies include the evaluation of new drug combinations with small molecules that may have improved efficacy compared with single agents. In addition to enhanced cytotoxicity, combinations of chemotherapeutic agents may inhibit and circumvent drug resistance. Among new therapeutic targets in ovarian cancer, the ET_AR represents an important receptor in the microecology of the tumor–host invasion field, capable of controlling tumor growth and progression by stimulating migration, invasiveness, and neovascularization and by promoting proliferation and survival (Bagnato and Rosanò 2008). Among several approaches for targeting the ET-1 axis in cancer therapy, ET_AR blockade with selective antagonists represents the most rational targeted approach in controlling the pleiotropic activities of ET-1, which are all pivotal in the gain and maintenance of ovarian malignant phenotype (Bagnato et al. 2008). Among various ET_AR antagonists, zibotentan is effectively capable of blocking specifically ET_AR-driven signalling pathways implicated in cancer cell proliferation and progression in vitro and of inducing tumor growth inhibition in ovarian cancer xenografts. Given the hypothesis that novel therapeutic agents in addition to combination regimens may be beneficial in ovarian cancer treatments, we previously analyzed in vitro and in vivo the antitumoral effects of zibotentan in combination with paclitaxel (Rosanò et al. 2007*b*). In an attempt to understand the biological mechanisms underlying the therapeutic effects of this combination therapy, we demonstrated that when the 2 agents were combined, a significant additive antitumor effect occurred, compared with a single regimen, that was associated with a dramatic inhibition of the pleiotropic events triggered by ET_AR activation. The increased commitment toward apoptosis was dependent on the activation of caspase-3 and poly(ADP-ribose) polymerase (PARP) and the inactivation of the antiapoptotic Bcl-2 protein, suggesting that zibotentan potentiates the antitumor activity of the cytotoxic agent paclitaxel by blocking survival pathways. The benefit of combining zibotentan with paclitaxel became more pronounced after in vivo treatment, with complete regression of tumors in 40% of treated mice. The therapeutic efficacy of the combination of zibotentan and paclitaxel was associated with a marked inhibition of tumor neovascularization and a reduction in the proliferation index, demonstrating that zibotentan enhances the antitumor effect of paclitaxel in vivo and also highlighting the potential benefit of combining 2 therapeutic agents that have entirely different mechanisms of action. These results complement and extend previous studies published by our group and others, and they support the concept that pharmacological blockade of the ET_AR using small molecule receptor antagonists in combination with cytotoxic drugs is therapeutically advantageous in different tumor types (Bagnato et al. 2008). The therapeutic efficacy of zibotentan, which has been shown to induce concomitant antitumor activity and inhibition of neovascularization, provides us with a rationale for the preclinical evaluation of this molecule in combination with different cytotoxic drugs. In agreement with previous results, in this study we found an increase in antitumor activity with the 3-way combination of zibotentan, cisplatinum, and paclitaxel that was associated with a significant suppression of tumor neovascularization and cell proliferation. In this context, the findings in different cancer cells that ET-1 acts as an antiapoptotic factor among downstream events after receptor activation indicate that this peptide may also modulate cell survival pathways through ET_AR-mediated PI3K-dependent Akt activation signaling. This suggests that activated ET_AR pathways can counteract the effect of chemotherapies, representing a valid target for anticancer therapy (Akhavan et al. 2006; Bagnato et al. 2008; Banerjee et al. 2007; Rosanò et al. 2007*a*, 2007*b*). Therefore, the combination of a specific ET_AR antagonist such as zibotentan with conventional chemotherapeutic drugs, such as the platinums and taxanes, would more effectively induce apoptosis, representing a more effective treatment strategy to be evaluated in the clinical setting.

Acknowledgements

We gratefully acknowledge the excellent study assistance of Stefano Masi, Valentina Caprara, and Aldo Lupo and the secretarial assistance of Maria Vincenza Sarcone. This study was funded in part by Associazione Italiana Ricerca sul Cancro, Italian Ministry of Health, and AstraZeneca (Macclesfield, UK).

References

Akhavan, A., McHugh, K.H., Guruli, G., Bies, R.R., Zamboni, W.C., Strychor, S.A., et al. 2006. Endothelin receptor A blockade enhances taxane effects in prostate cancer. Neoplasia, **8**(9): 725–732. doi:10.1593/neo.06388. PMID:16984730.

Bagnato, A., and Rosanò, L. 2008. The endothelin axis in cancer. Int. J. Biochem. Cell Biol. **40**(8): 1443–1451. doi:10.1016/j.biocel.2008.01.022. PMID:18325824.

Bagnato, A., Tecce, R., Moretti, C., Di Castro, V., Spergel, D., and Catt, K.J. 1995. Autocrine actions of endothelin-1 as a growth factor in human ovarian carcinoma cells. Clin. Cancer Res. **1**(9): 1059–1066. PMID:9816080.

Bagnato, A., Salani, D., Di Castro, V., Wu-Wong, J.R., Tecce, R., Nicotra, M.R., et al. 1999. Expression of endothelin 1 and en-

dothelin A receptor in ovarian carcinoma: evidence for an autocrine role in tumor growth. Cancer Res. **59**(3): 720–727. PMID: 9973223.

Bagnato, A., Spinella, F., and Rosanò, L. 2005. Emerging role of the endothelin axis in ovarian tumor progression. Endocr. Relat. Cancer, **12**(4): 761–772. doi:10.1677/erc.1.01077. PMID: 16322321.

Bagnato, A., Spinella, F., and Rosanò, L. 2008. The endothelin axis in cancer: the promise and the challenges of molecularly targeted therapy. Can. J. Physiol. Pharmacol. **86**(8): 473–484. doi:10.1139/Y08-058. PMID:18758494.

Banerjee, S., Hussain, M., Wang, Z., Saliganan, A., Che, M., Bonfil, D., et al. 2007. In vitro and in vivo molecular evidence for better therapeutic efficacy of ABT-627 and taxotere combination in prostate cancer. Cancer Res. **67**(8): 3818–3826. doi:10.1158/0008-5472.CAN-06-3879. PMID:17440096.

Bast, R.C., Jr., Hennessy, B., and Mills, G.B. 2009. The biology of ovarian cancer: new opportunities for translation. Nat. Rev. Cancer, **9**(6): 415–428. doi:10.1038/nrc2644. PMID:19461667.

Del Bufalo, D., Di Castro, V., Biroccio, A., Varmi, M., Salani, D., Rosanò, L., et al. 2002. Endothelin-1 protects ovarian carcinoma cells against paclitaxel-induced apoptosis: requirement for Akt activation. Mol. Pharmacol. **61**(3): 524–532. doi:10.1124/mol.61.3.524. PMID:11854432.

Growcott, J.W. 2009. Preclinical anticancer activity of the specific endothelin A receptor antagonist ZD4054. Anticancer Drugs, **20**(2): 83–88. doi:10.1097/CAD.0b013e328320791c. PMID: 19065106.

Hennessy, B.T., Coleman, R.L., and Markman, M. 2009. Ovarian cancer. Lancet, **374**(9698): 1371–1382. doi:10.1016/S0140-6736(09)61338-6. PMID:19793610.

Levin, E.R. 1995. Endothelins. N. Engl. J. Med. **333**(6): 356–363. doi:10.1056/NEJM199508103330607. PMID:7609754.

Masaki, T. 2000. The endothelin family: an overview. J. Cardiovasc. Pharmacol. **35**(4 Suppl 2): S3–S5. doi:10.1097/00005344-200000002-00002. PMID:10976772.

Morbidelli, L., Orlando, C., Maggi, C.A., Ledda, F., and Ziche, M. 1995. Proliferation and migration of endothelial cells is promoted by endothelins via activation of ET_B receptors. Am. J. Physiol. **269**(2 Pt 2): H686–H695. PMID:7653633.

Morris, C.D., Rose, A., Curwen, J., Hughes, A.M., Wilson, D.J., and Webb, D.J. 2005. Specific inhibition of the endothelin A receptor with ZD4054: clinical and pre-clinical evidence. Br. J. Cancer, **92**(12): 2148–2152. doi:10.1038/sj.bjc.6602676. PMID: 15956965.

Nelson, J.B., Udan, M.S., Guruli, G., and Pflug, B.R. 2005. Endothelin-1 inhibits apoptosis in prostate cancer. Neoplasia, **7**(7): 631–637. PMID:16026642.

Rosanò, L., Spinella, F., Di Castro, V., Nicotra, M.R., Dedhar, S., de Herreros, A.G., et al. 2005. Endothelin-1 promotes epithelial-to-mesenchymal transition in human ovarian cancer cells. Cancer Res. **65**(24): 11649–11657. doi:10.1158/0008-5472.CAN-05-2123. PMID:16357176.

Rosanò, L., Di Castro, V., Spinella, F., Decandia, S., Natali, P.G., and Bagnato, A. 2006. ZD4054, a potent endothelin receptor A antagonist, inhibits ovarian carcinoma cell proliferation. Exp. Biol. Med. (Maywood), **231**(6): 1132–1135. PMID:16741063.

Rosanò, L., Di Castro, V., Spinella, F., Tortora, G., Nicotra, M.R., Natali, P.G., and Bagnato, A. 2007*a*. Combined targeting of endothelin A receptor and epidermal growth factor receptor in ovarian cancer shows enhanced antitumor activity. Cancer Res. **67**(13): 6351–6359. doi:10.1158/0008-5472.CAN-07-0883. PMID:17616694.

Rosanò, L., Di Castro, V., Spinella, F., Nicotra, M.R., Natali, P.G., and Bagnato, A. 2007*b*. ZD4054, a specific antagonist of the endothelin A receptor, inhibits tumor growth and enhances paclitaxel activity in human ovarian carcinoma in vitro and in vivo. Mol. Cancer Ther. **6**(7): 2003–2011. doi:10.1158/1535-7163.MCT-07-0151. PMID:17620430.

Salani, D., Di Castro, V., Nicotra, M.R., Rosanò, L., Tecce, R., Venuti, A., et al. 2000*a*. Role of endothelin-1 in neovascularization of ovarian carcinoma. Am. J. Pathol. **157**(5): 1537–1547. PMID: 11073813.

Salani, D., Taraboletti, G., Rosanò, L., Di Castro, V., Borsotti, P., Giavazzi, R., and Bagnato, A. 2000*b*. Endothelin-1 induces an angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in vivo. Am. J. Pathol. **157**(5): 1703–1711. PMID:11073829.

Shichiri, M., Kato, H., Marumo, F., and Hirata, Y. 1997. Endothelin-1 as an autocrine/paracrine apoptosis survival factor for endothelial cells. Hypertension, **30**(5): 1198–1203. PMID:9369276.

Weidner, N., Folkman, J., Pozza, F., Bevilacqua, P., Allred, E.N., Moore, D.H., et al. 1992. Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. J. Natl. Cancer Inst., **84**(24): 1875–1887. PMID:1281237.