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Combinations of Platinums and Selected Phytochemicals as a Means of Overcoming Resistance in Ovarian Cancer

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Abstract. *Cancer sufferers are often found to use herbal products along with targeted therapy although not much information (whether beneficial or harmful) is available about the effects of such combinations. In this study, we investigated synergism from the combination of platinum drugs and a number of tumour-active phytochemicals including curcumin, epigallocatechin-3-gallate, thymoquinone, genistein, resveratrol, betulinic acid and ursolic acid in three human ovarian cancer cell lines A2780, A2780^{cisR} and A2780^{ZD0473R}, as a function of concentration and the sequence of administration. Both the dose–effect curves and combination indices show that the binary combinations of platinum drugs with the phytochemicals exert concentration- and sequence-dependent synergism in the cell lines. Generally the degree of synergism is found to be greater in sequenced administration such as 0/2 h, 2/0 h, 0/4 h and 4/0 h than the bolus. The variation in the nature of the combined drug action from being highly synergistic to antagonistic with the change in sequence of administration clearly indicates that the action of one drug modulates that of the other (towards the induction or inhibition of apoptosis). We have also used sequenced combinations of platinum drugs and bortezomib (a proteasome inhibitor that prevents cisplatin-induced proteasomal degration of copper transporter CTR1) to enhance cellular platinum accumulation and the level of platinum–DNA binding especially in the resistant human ovarian tumour models. Proteomic studies to identify the key proteins associated with platinum resistance are ongoing. We have identified 59 proteins associated with platinum resistance in ovarian tumor models.*

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High mortality rate from ovarian cancer remains an on-going challenge (1) mainly because of the development of drug resistance (2-4). In an attempt to overcome platinum resistance in ovarian and other types of cancers, many cisplatin analogues and rule-breaker platinum compounds have been prepared elsewhere and in our laboratory. Figure 1 gives the structures of some of the compounds that have been synthesized by us. Some of the compounds such as YH12, DH6Cl and QH1 have much higher activity than cisplatin, especially against cisplatin-resistant ovarian tumor models.

Recently, there has been a growing interest in the use of dietary chemopreventive agents (such as phytochemicals) in combination with chemotherapeutics towards the inhibition of cancer cell growth. Since tumour-active dietary compounds generally exert their anti-tumour activity through the regulation of cell signaling pathways different from those of platinum drugs, it is logical to believe that platinum drugs may act synergistically in combination with the dietary compounds. Such synergistic combinations may also reduce the systemic toxicity caused by chemotherapies or radiotherapies because of lower required doses (5). It may be noted that the major factors involved in the development of drug resistance are multidrug resistance gene, nuclear factor-κB (*NF-κB*), and serine/threonine protein kinase AKT. About 15% of all solid tumours are found to be driven by NF-κB as a player, whereas most cancer-preventive agents are believed to be NF-κB inhibitors (6). The acquired resistance to cisplatin in ovarian cancer has been shown to be linked to the activation of NF-κB, whereas the chemo-sensitization of ovarian cancer cells due to combination of cisplatin with phytochemicals such as genistein, curcumin and resveratrol are believed to be due to inactivation of NF-κB (6). As a part of our continued studies seeking ways to overcome drug resistance, we applied sequenced combinations of targeted therapy and a number of selected tumor-active phytochemicals including epigallocatechin-3-gallate, capsaicin, genistein, curcumin, quercetin, resveratrol and

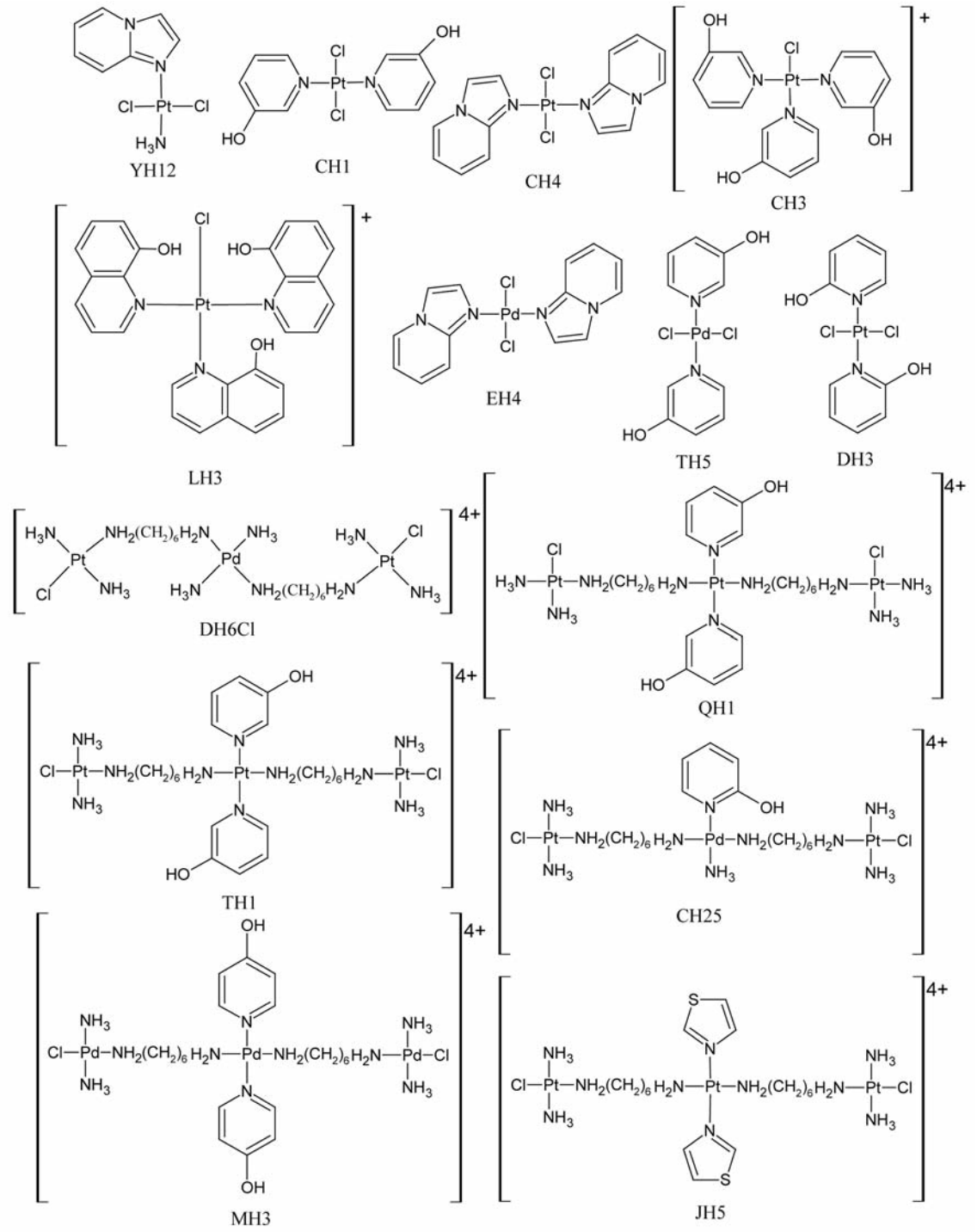

Figure 1. *Chemical structures of YH12, CH1, CH4, CH3, LH3, EH4, TH5, DH3, DH6Cl, QH1, TH1, CH25, MH3 and JH5.*

thymoquinone that are well-known anti-oxidants and display a variety of biological activities, including chemoprevention and inhibition of tumor growth (6). Quercetin exerts its anti-tumour activity by inhibiting the activation of NF-κB (7). For example, the phytochemical resveratrol inhibits cell proliferation, induces apoptosis and overcomes chemoresistance through the down-regulation of NF-κB, signal transducer and activator of transcription-3

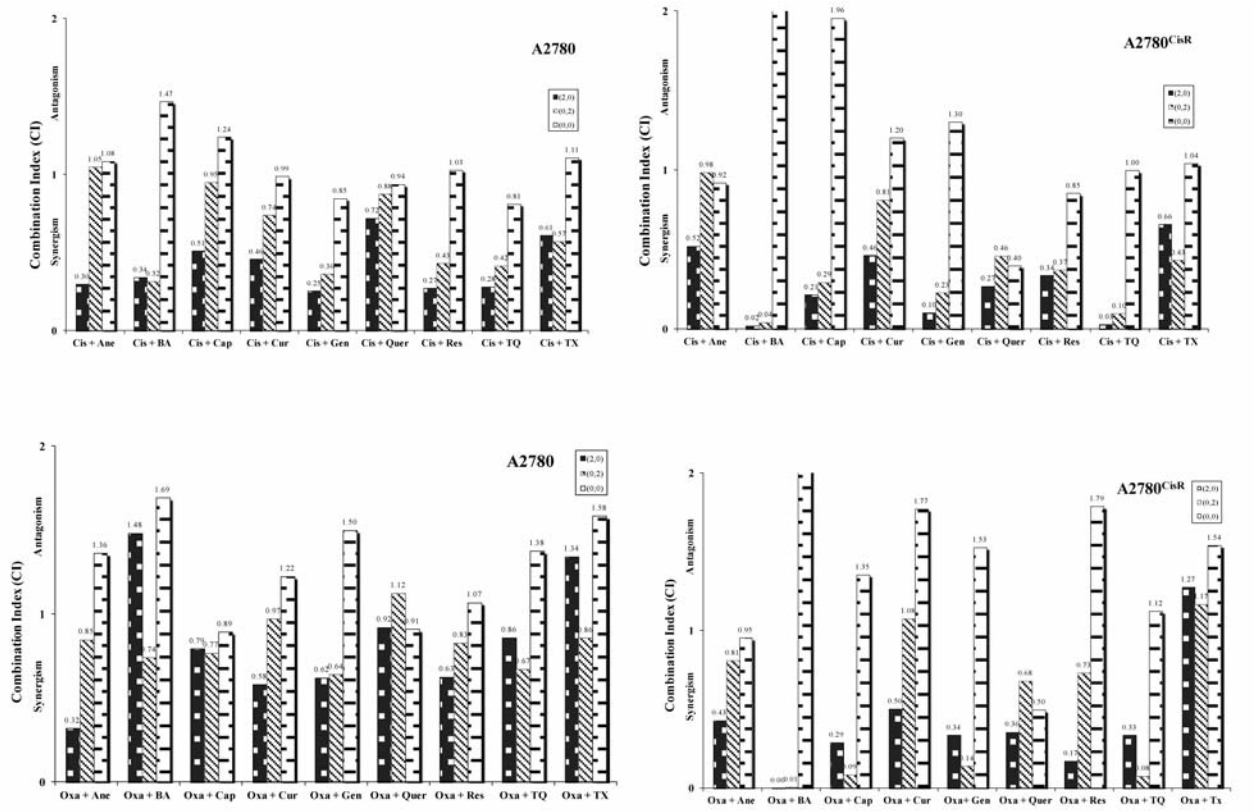

Figure 2. *Combination indices at median-effect dose applying to the combinations of cisplatin (Cis) and oxaliplatin (Oxa) with a number of phytochemicals in A2780 and A2780cisR cell lines, indicating generally administrations with 2 h time gap are synergistic whereas bolus is additive to antagonistic: CI >1: synergistic; =1: additive and >1: antagonistic.*

(STAT3), and anti-apoptotic and cell survival gene products (8), and also by regulating cyclooxygenase expression (9). It also up-regulates the tumour-suppressor p53 and the expression of cytokine (MIC-1) that possesses anti-tumourigenic activity (10). Thymoquinone exerts anti-inflammatory effects and inhibits tumour cell proliferation through modulation of apoptosis signaling, inhibition of angiogenesis, and cell-cycle arrest (5). The compound is associated with an increased expression of p53 and the downstream p53 target gene, $p21^{WAF1}$. The apoptotic effect of thymoquinone is also modulated by the apoptosis B-cell lymphoma (BCL2) protein (11). In addition, it acts as an inhibitor of angiogenesis within cells (12). Curcumin also causes cancer cell death through apoptosis. The compound is believed to target multiple molecular targets such as pro-apoptotic proteins including p53 and bax, transcription factors including NF-κB, AKT, p38 mitogen-activated protein kinases, cytokines including growth factors such as epidermal growth factor receptor and platelet-derived growth factor. From the above, it is logical to expect that the combination of platinum drugs such as cisplatin and oxaliplatin with phytochemicals may exhibit sequence-dependent synergism (13-15).

Results and Discussion

Combination indices at median-effect dose applying to the combinations of cisplatin (Cis) and oxaliplatin (Oxa) with phytochemicals anethole (Ane), betulinic acid (BA), capscaicin (Cap), curcumin (Cur), genistein (Gen), quercetin (Quer), resveratrol (Res), thymoquinone (TQ) and taxol (Tx) in A2780 and $A2780^{cisR}$ cell lines, indicating that generally administrations with 2-h time gap are synergistic, whereas bolus is additive to antagonistic (CI >1: synergistic; =1: additive and >1: antagonistic) (Figure 2).

We also carried out proteomic studies based on 2D gel electrophoresis and mass spectrometry to characterize key proteins associated with platinum resistance in $A2780^{cisR}$ ovarian tumour model as compared to cisplatin-responsive A2780 ovarian tumour model. A total of 59 proteins associated with platinum resistance were been identified. Based on the functions and sub-cellular locations proteins

those had been identified from 2D-gel are divided into the following six major groups: (I) Cytoskeletal proteins involved in invasion and metastasis: profilin 1, stathmin 1, cofilin-2, microtubule-associated protein RP/EB family member 1, Actin cytoplasmic 1, tubulin beta chain, alpha-enolase, liver-enriched gene 1and vimentin; (II) Molecular chaperones and stress-related proteins: peptidyl-prolyl *cis-trans* isomerase A, 60 kDa heat shock protein, T-complex protein 1 subunit theta, T-complex protein 1, heat shock protein 7C, 75 kDa glucose-regulated protein, 78 kDa glucose-regulated protein, endoplasmin, heat shock 70 kDa protein 4, calumenin, protein disulfide-isomerase A1, protein disulfide-isomerase A3, stress-induced-phosphoprotein 1, transitional endoplasmic reticulum ATPase , hypoxia up-regulated protein 1, calreticulin, peptidyl-prolyl cis-trans isomerase FKBP4, heat shock protein HSP 90-beta , heat shock protein beta-1 and ubiquitin carboxyl-terminal hydrolase isozyme L1; (III) Proteins involved in detoxification and drug resistance: peroxiredoxin-1, Annexin A5, Annexin A1, peroxiredoxin-6, glutathione S-transferase P and phosphoglycerate kinase 1; (IV) Proteins involved in metabolic processes: triosephosphate isomerase, L-lactate dehydrogenase B chain, 40S ribosomal protein SA (RSSA), cytochrome *c* oxidase subunit 5A, ATP synthase subunit alpha (ATPA), actin cytoplasmic 1, serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform, inositol-3-phosphate synthase 1 and glyceraldehyde-3-phosphate dehydrogenase; (V) mRNA processing proteins: heterogeneous nuclear ribonucleoprotein F (HNRPF), heterogeneous nuclear ribonucleoprotein K and heterogeneous nuclear ribonucleoprotein C, and (VI) Others: nuclear autoantigenic sperm protein, 14-3-3 protein gamma, nucleophosmin, adenosylhomocysteinase, ruvB-like 2, glucosidase 2 subunit beta, nucleoside diphosphate kinase A, protein DJ-1, GTP- binding nuclear protein Ran and ran-specific GTPase-activating protein.

Among the identified proteins, those recognized to play a role in drug resistance in ovarian cancer were: cytochrome *c* oxidase subunit 5A, profilin 1, stathmin, cofilin-2, microtubule-associated protein RP/EB family member 1, Annexin A5, Annexin A1, 40S ribosomal protein SA, actin cytoplasmic 1, calumenin, heterogeneous nuclear ribonucleoprotein F, ATP synthase subunit beta, ATP synthase subunit alpha, tubulin beta chain, protein disulfide-isomerase A3, vimentin, heterogeneous nuclear ribonucleoprotein K, 60 kDa heat shock protein (CH60), T-complex protein 1 subunit theta, protein disulfide-isomerase A3 (PDIA1), T-complex protein 1 subunit alpha, nuclear autoantigenic sperm protein, heat shock cognate 71 kDa protein, heat shock cognate 71 kDa protein, 78 kDa glucose-regulated protein, endoplasmin heat shock protein 90 kDa beta member 1, heat shock 70 kDa protein 4, peroxiredoxin-6 and heterogeneous nuclear ribonucleoproteins C1/C2.

Conclusion

Combinations of platinum drugs and selected tumour-active phytochemicals are found to show sequenced dependent synergism in ovarian tumour models. If conformed *in vivo*, the results may be highly significant clinically.

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References

1 Gallion HH, Pieretti M, DePriest PD and van Nagell JR Jr.: The molecular basis of ovarian cancer. Cancer *76*: 1992-1997, 1995.
2 Kartalou M and Essigmann JM: Mechanisms of resistance to cisplatin. Mut Res *478*: 23-43, 2001.
3 Loehrer PJ and Einhorn LH: Diagnosis and treatment: Drugs five years later. cisplatin. Ann Int Med *100(5)*: 704-713, 1984.
4 Pinedo HM and Schornagel JH: Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy. Plenum Press: New York, 1996.
5 Chan MM and Fong D: Overcoming ovarian cancer drug resistance with phytochemicals and other compounds. Prog Cancer Drug Resist Res 1-28, 2007.
6 Sarkar FH, Li Y, Sarkar FH and Li Y: Using chemopreventive agents to enhance the efficacy of cancer therapy. Cancer Res *66*: 3347-3350, 2006.
4 Banerjee S, Padhye S, Azmi A, Wang Z, Philip PA, Kucuk O, Sarkar FH and Mohammad RM: Review on molecular and therapeutic potential of thymoquinone in cancer. Nutr Cancer *62*: 938-946, 2010.
5 Solomon Leigh A, Ali S, Banerjee S, Munkarah Adnan R, Morris Robert T and Sarkar Fazlul H: Sensitization of ovarian cancer cells to cisplatin by genistein: the role of NF-κB. J Ovarian Res 1, 9, 2008.
6 Chan MM, Fong D, Soprano KJ, Holmes WF, Heverling H, Chan MM, Fong D, Soprano KJ, Holmes WF and Heverling H: Inhibition of growth and sensitization to cisplatin-mediated killing of ovarian cancer cells by polyphenolic chemopreventive agents. J Cell Physiol *194*: 63-70, 2003.
7 Aggarwal BB and Shishodia S: Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol *71*: 1397-1342, 2006.
8 Hu Y, Rahlfs S, Mersch-Sundermann V, Becker K, Hu Y, Rahlfs S, Mersch-Sundermann V and Becker K: Resveratrol modulates mRNA transcripts of genes related to redox metabolism and cell proliferation in non-small-cell lung carcinoma cells. Biol Chem *388*: 207-219, 2007.
9 Sexton E, Van Themsche C, LeBlanc K, Parent S, Lemoine P, Asselin E, Sexton E, Van Themsche C, LeBlanc K, Parent S, Lemoine P and Asselin E: Resveratrol interferes with AKT activity and triggers apoptosis in human uterine cancer cells. Mol Cancer *5*: 45, 2006.
10 Golkar L, Ding XZ, Ujiki MB, Salabat MR, Kelly DL, Scholtens D, Fought AJ, Bentrem DJ, Talamonti MS, Bell RH, Adrian TE, Golkar L, Ding X-Z, Ujiki MB, Salabat MR, Kelly DL,

Scholtens D, Fought AJ, Bentrem DJ, Talamonti MS, Bell RH and Adrian TE: Resveratrol inhibits pancreatic cancer cell proliferation through transcriptional induction of macrophage inhibitory cytokine-1. J Surg Res *138*: 163-169, 2007.

11 Gali-Muhtasib H, Diab-Assaf M, Boltze C, Al-Hmaira J, Hartig, R, Roessner A, Schneider-Stock R, Gali-Muhtasib H, Diab-Assaf M, Boltze C, Al-Hmaira J, Hartig R, Roessner A and Schneider-Stock R: Thymoquinone extracted from black seed triggers apoptotic cell death in human colorectal cancer cells *via* a p53-dependent mechanism. Int J Oncol *25*: 857-866, 2004.

12 Gali-Muhtasib H, Roessner A, Schneider-Stock R, Gali-Muhtasib H, Roessner A and Schneider-Stock R: Thymoquinone: a promising anti-cancer drug from natural sources. Int J Biochem Cell Biol *38*: 1249-1253, 2006.

13 Yunos Nurhanan M, Beale P, Yu Jun Q and Huq F: Synergism from Sequenced Combinations of Curcumin and Epigallocatechin-3-gallate with Cisplatin in the Killing of Human Ovarian Cancer Cells. Anticancer Res *31*: 1131-1140, 2011.

14 Alshehri A, Beale P, Yu JQ, Huq F, Alshehri A, Beale P, Yu JQ, and Huq F: Synergism from combination of cisplatin and a trans-platinum compound in ovarian cancer cell lines. Anticancer Res *30*: 4547-4553, 2010.

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