Epigallocatechin Gallate Acts Synergistically in Combination with Cisplatin and Designed *trans*-palladiums in Ovarian Cancer Cells

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Abstract. *In this study, synergism in activity from the sequenced combinations of three trans-palladiums (denoted as TH5, TH6 and TH7) with green tea polyphenol (−)-epigallocatechin-3-gallate (EGCG), as well as that with cisplatin, was investigated in a number of human ovarian tumour models as a function of sequence of administration. Cellular accumulation of platinum and palladium, and the levels of platinum−DNA and palladium−DNA binding were also determined for the 0/4 h and 0/0 h sequences of administration. The results of the study show that co-administration of cisplatin with EGCG (0/0 h) produces weak synergism in both cisplatin-sensitive (A2780) and cisplatin-resistant (A2780^{cisR}) cell lines whereas (0/4 h) administration produces pronounced synergism in both. In contrast, bolus administration of EGCG with TH5, TH6 and TH7 produces marked antagonism except that with TH5, in the A2780^{cisR} cell line, where a mild synergism is observed. In the case of TH5, TH6 and TH7, administration of drugs with a time gap (0/4 h or 4/0 h combinations) produces sequence-dependent synergism in both A2780 and A2780^{cisR} cell lines, whereas in the case of cisplatin, marked antagonism is observed with the 4/0 h sequence of administration in the A2780 cell line. Whereas the highly synergistic 0/4 h sequence of combination of cisplatin with EGCG is found to be associated with pronounced cellular accumulation of platinum and a high level of platinum−DNA binding, no such clear trend can be seen for any of the combinations of TH5, TH6 and TH7 with EGCG. The results of the present study provide support to the idea that sequenced combinations of platinum drugs and tumour-active palladium compounds with selected phytochemicals such as EGCG may provide a means of overcoming drug resistance.*

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Epithelial ovarian cancer, the most lethal form of gynaecological malignancy, affects more than two-hundred thousand women each year around the world (1). The high lethality is due to poor prognosis, the early asymptomatic character, and the heterogenic nature of the tumour (2). Most often ovarian cancer is diagnosed at an advanced stage when tumours have spread beyond the ovaries in approximately two-thirds of the patients (3). Although the combination of cisplatin (or carboplatin) and paclitaxel are the standard choice of chemotherapy for ovarian cancer, platinum resistance (acquired or intrinsic) remains a major problem in the treatment of epithelial ovarian cancer (4). Therefore, new drugs and different regimens consisting of combinations of chemotherapeutic agents are needed in order to improve the therapeutic window of platinum-based drugs. In recent years, palladium-based compounds have emerged as an attractive new class of potent anticancer drugs (5). Indeed a number of *trans*-palladium compounds having bulky planaramine ligands has been found to display significant cytotoxicity towards different cancer cell lines, including cisplatin-resistant ovarian cancer cell lines (6-9). Unlike cisplatin which mainly forms intrastrand adducts with DNA, *trans*-palladium complexes are likely to form mainly interstrand DNA adducts that would cause more of a global change in the DNA conformation. The difference in the nature of interaction with DNA may explain why a number of *trans*-palladium complexes have been found to show greater cytotoxicity towards cisplatin-resistant ovarian cancer cell lines (10).

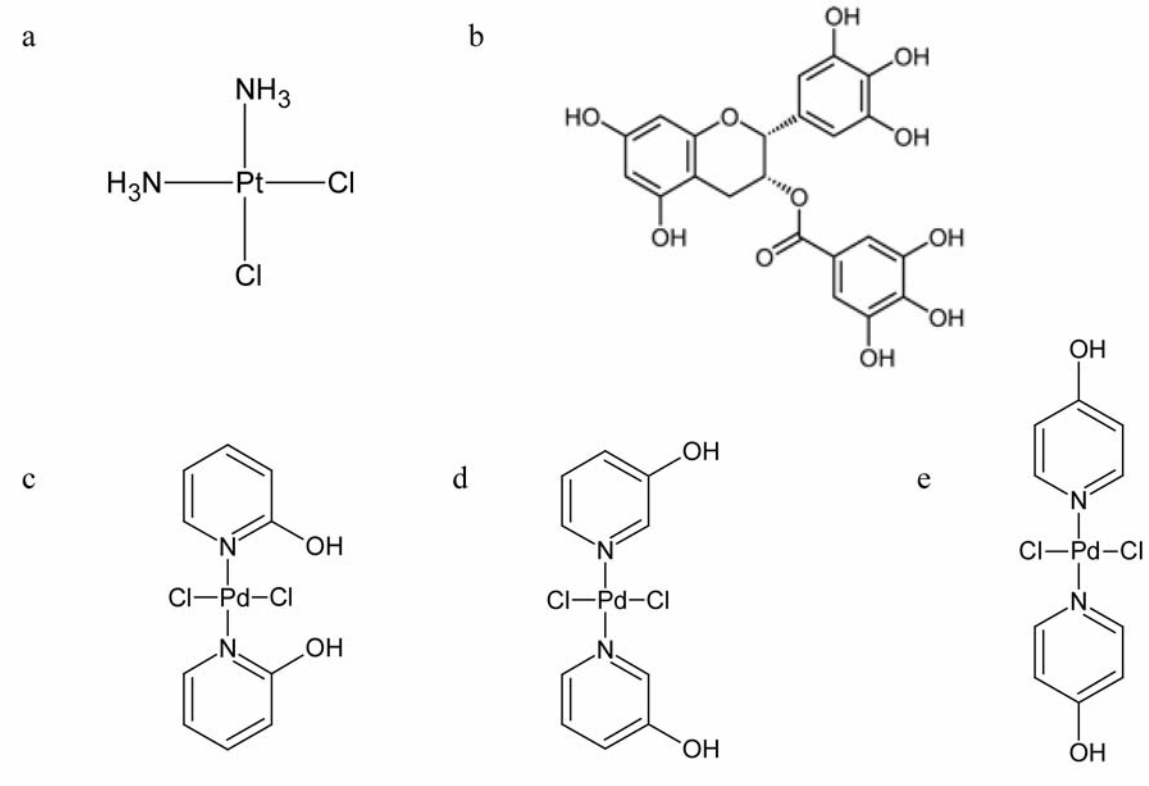

Figure 1. *Structures of cisplatin (a), (−)-epigallocatechin-3-gallate (b), TH5 (c), TH6 (d), and TH7 (e).*

Combination of innocuous dietary phytochemicals with anticancer drugs is a promising new strategy for increasing the antitumour response of chemotherapeutics (11). Phytochemicals can sensitize tumour cells towards chemotherapeutic agents by modulating various cellular pathways involved in chemoresistance (12-14). One such phytochemical is (−)-epigallocatechin-3-gallate (EGCG), which is the key active component of green tea, commonly consumed as a cancer chemopreventive (15). Growing evidence demonstrates that EGCG has a diverse range of effects including antioxidant, anticarcinogenic, antiangiogenic, antitumour and, more importantly, as a modulator of tumour cell response to chemotherapy (16, 17). For example, the combination of EGCG with different anticancer agents was found to enhance the induction of apoptosis in PC-9 cells by a factor of 10-15 (18, 19). Our previous results also demonstrated that more pronounced killing of human ovarian cancer cells can be achieved from the sequenced administration of cisplatin and EGCG (20). Since *trans*-palladium compounds may be sufficiently tumour active and EGCG can sensitize tumour cells to chemotherapeutic agents, we hypothesized that combination of *trans*-palladiums with EGCG may also act synergistically. In this study, we aimed at investigating the effects of sequenced combination of cisplatin and three tumour-active *trans*-palladium compounds: *trans-bis*(3-hydroxypyridine)dichloropalladium(II), *trans-bis*(2-hydroxypyridine)dichloropalladium(II), and *trans-bis*(4-hydroxypyridine)dichloropalladium(II), respectively coded as TH5, TH6 and TH7 (Figure 1) with EGCG in both cisplatin-sensitive and cisplatin-resistant ovarian cancer cells. To the best of our knowledge, this is the first report of the combination study between *trans*-palladium compounds and EGCG in cancer cell lines.

Materials and Methods

Drugs. Cisplatin was prepared following Dhara's method (21). The three *trans*-palladiums, TH5, TH6 and TH7 were prepared according to previously-described methods (6, 22). EGCG was obtained in powder form Sigma–Aldrich Sydney, Australia. Cisplatin, TH5, TH6 and TH7 were initially dissolved in dimethyl-formamide (DMF) followed by the addition of milliQ water (at a ratio 1:5) to give 1 mM stock solution. EGCG was dissolved in ethanol to give 1 mM solution. The solutions were filtered using DISMIC-25cs ADVANTEC filter (cellulose acetate, 0.20 μm hydrophilic, pressure limitation: 0.51 MPa; Millipore, Ireland) to sterilize. The stock solutions were serially diluted with freshly-prepared Roswell Park Memorial Institute-1640 (RPMI-1640) medium to produce a range of final concentrations from 0.08 to 100 μM for cisplatin, TH5, TH6 and TH7.

Cell lines. A2780 (cisplatin-sensitive, parental cell line), $A2780^{cisR}$ (cisplatin-resistant) and $A2780^{ZD0473R}$ (ZD0473-resistant) ovarian

cancer cell lines and multi-drug resistant SKOV-3 [Sloan-Kettering human epidermal growth factor receptor 2 (HER2) 3+ ovarian cancer cell line] were obtained from Ms. Zhang from Royal Prince Alfred Hospital, University of Sydney, Australia. The cell lines were sub-cultured in RPMI–1640 media that were prepared with 10% fetal calf serum (FCS), 1 mM Hepes, 5.6% sodium bicarbonate and 200 mM glutamine.

Cytotoxicity assays. The cell kill due to dugs-alone and in combination were determined using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay (23). Briefly, 4000 to 6000 cells per well in RPMI-1640 medium were seeded into flat-bottomed 96-well culture plates and allowed to attach overnight. For single-treatments, drugs were added at a range of at least three to five different concentrations to triplicate wells and left in an incubator (at 37˚C, with 5% carbon dioxide in air, at pH 7.4) for 72 h. After preparation of serial five-fold dilutions of the drugs in 10% FCS/RPMI-1640 medium (0.08 to 20 μM for cisplatin and 0.80 to 100 μM for TH5, TH6 and TH7), 100 μl of drugs were added to equal volumes of cell culture in triplicate wells and left to incubate under normal growth conditions for 72 h, at 37˚C in a humidified atmosphere. For combination studies, cells were treated with increasing concentrations of drugs at constant ratios of their inhibitory concentration 50 *i.e*. drug concentrations required for 50% cell kill (IC_{50}) values, using the sequences: 0/0 h, 0/4 h and 4/0 h, where 0/0 h means that both the drugs were added at the same time, 0/4 h means that cisplatin or TH5/TH6/TH7 was added first, followed by the addition of EGCG 4 h later, and 4/0 h means the converse. The concentration ranges were: cisplatin: 0.08-1.25 μM and 0.99-15.87 μM; EGCG: 1.37-21.98 μM and 1.33-21.34 μM; TH5: 3.54-56.67 μM and 2.73-43.65 μM; TH6: 0.87-13.86 μM and 0.89-14.30 μM; TH7: 2.71-43.37 μM and 2.39-38.18 μM for A2780 and A2780^{cisR} cell lines, respectively. At the completion of the 72 h incubation period, the MTT assay was performed, as in previous experiments (23).

Combined drug action study. The combined action of the drugs was investigated using the median-effect analysis. The combination index (CI) used as a quantitative measure of combined action was calculated based on the pooled data from three to five individual experiments each with at least three data points for each drug-alone and for the drug combinations. The CI for two drugs in combination was calculated using the following equation (24, 25).

$$CI = \frac{D_1}{D_{1x}} + \frac{D_2}{D_{2x}} \qquad \text{Eqtn. 1}$$

where D_1 and D_2 in the numerator are the concentrations of compounds 1 and 2 in combination needed to achieve x% inhibition, whereas D_{1x} and D_{2x} in the denominator are those required when the drugs are present-alone. In the following equation, D_x denotes the dose of drug, D_m is the median-effect dose meaning the dose required for 50% cell kill, f_a is the fraction of cells affected (*i.e*. killed) by the dose, f_u is the fraction of cells remaining unaffected so that $f_u=1-f_a$ and m is the exponent defining the shape of the dose effect curve. Three notations IC_{50}, D_m and ED_{50} are commonly used to indicate the median effect dose (26). Whereas use of IC_{50} is more common for a single drug, D_m is more commonly used for drug combinations. Also, the notations IC_{50} and ED_{50} in essence indicate the same, except that IC_{50} implies dose required for 50% cell kill whereas ED_{50} indicates dose required to achieve median effect. This means ED_{50} is a more general term than IC_{50}.

$$D_x = D_m[f_a/(1-f_a)]^{1/m} \qquad \text{Eqtn. 2}$$

A CI of <1, =1 and >1 indicates synergism, additivity and antagonism, respectively, in the combined drug action. D_m indicates the median effect dose that corresponds to the IC_{50} value. The linear correlation co-efficient, 'r' indicates the goodness-of-fit for the pooled data (where r=1 is a perfect fit). The linear correlation co-efficient, r, of the median effect plot for the cell culture system should be greater than 0.95 (r>0.95). The CI, D_m and r values were calculated using Calcusyn software (V2) (Biosoft, UK).

Platinum/palladium accumulation and platinum–DNA or palladium–DNA binding. Since an essential step in the cell kill due to platinum and palladium drugs is believed to be their binding with DNA, the most synergistic (0/4 h) and least synergistic (0/0 h) combinations of cisplatin with EGCG were selected for the determination of cellular platinum accumulation and platinum–DNA binding. Similarly, for TH5, TH6 and TH7 with EGCG, the most synergistic (0/4 h) and most antagonistic (0/0 h) combinations were selected for the determination of cellular palladium accumulation and palladium–DNA binding. It is believed that the results may aid in the understanding of the combined drug action. The method used for the determination of total intracellular platinum, palladium, platinum–DNA, as well as palladium–DNA levels, was a modification of a previously described method (27). Cisplatin, palladium drugs and EGCG were added to culture plates containing exponentially growing A2780 and A2780^{cisR} cells in 5 ml 10% FCS/RPMI-1640 culture medium (cell density=1×10^6 cells ml). The cells containing the drugs were incubated for 24 h, at the end of which cell monolayers were trypsinized and cell suspension (5 ml) was transferred to centrifuge tubes and spun at 2041 ×g using a Beckman CS-15R centrifuge, for 2 min at 4˚C. The cells were washed twice with ice-cold phosphate-buffered saline (PBS) and the pellets were stored at –20˚C until assayed.

Cellular accumulation. Cell pellets from cells treated with drug combinations were suspended in 0.5 ml 1% triton-X, held on ice while being sonicated. Total intracellular platinum and palladium contents were determined by graphite furnace atomic absorption spectrophotometer (AAS) using a Varian SpectrAA-240 plus with Graphite Tube Atomizer (GTA) 120 AAS (Varian Australia Pty Ltd, Victoria, Australia) (28).

Platinum–DNA and palladium–DNA binding. DNA was isolated from the cell pellet using H440050 JETQUICK Blood DNA Spin Kit/50 (Austral Scientific Pty Ltd, New South Wales, Australia) according to the modified protocol of Bowtell (29). DNA content was determined by UV spectrophotometry (260 nm) (Varian Cary 1E UV-Visible with Varian Cary Temperature Controller, Varian Australia Pty Ltd, Victoria, Australia) and platinum and palladium levels were determined by graphite furnace AAS. A_{260}/A_{280} ratios were found to be between 1.75 and 1.8 for all samples, indicating their high purity (30), and the DNA concentration was calculated according to the equation:

Concentration=Absorbance at 260 nm × 50 ng/μl.

Table I. *Inhibitory concentration 50 (IC_{50})[a] values (μM) and resistance factors (RF) for cisplatin, the trans-palladiums TH5, TH6, TH7 and EGCG, as applied to the human ovarian cancer cell lines A2780, A2780^{cisR}, A2780^{ZD0473R} and SKOV-3.*[b]

Drug	A2780	A2780^{cisR}	RF[c]	A2780^{ZD0473R}	RF[d]	SKOV-3
TH5	17.71±4.67	13.64±5.91	0.77	12.50±2.12	0.71	14.34±2.72
TH6	4.33±2.19	4.47±2.08	1.03	4.92±2.49	1.13	3.36±0.95
TH7	13.55±3.41	11.93±2.20	0.88	12.31±1.96	0.90	43.91± 10.24
EGCG	6.87±2.72	6.67±3.61	0.97	9.63±4.73	1.40	11.08±1.21
Cis	0.39±0.09	4.96±2.60	12.72	4.99±2.06	12.79	1.45±0.55

[a]The IC_{50} values were obtained from the results of quadruplicate determinations of at least three independent experiments, and are given as means±standard deviation (SD). [b]The results are averages of those obtained from four identical wells with 4000 cells per well. [c]Ratio of IC_{50} value for A2780^{cisR} to A2780 cell line. [d]Ratio of IC_{50} value for A2780^{ZD0473R} to A2780 cell line.

Table II. *Dose-effect parameters applying to combinations of (−)-epigallocatechin-3-gallate (EGCG) with cisplatin (Cis) and trans-palladiums (TH5, TH6 and TH7) in the A2780 and A2780^{cisR} cell lines.*

Drug	Sequence (h)	A2780					A2780^{cisR}				
		Molar ratio	CI at ED_{50}	D_m	m	r	Molar ratio	CIs at ED_{50}	D_m	m	r
Cis				0.36	0.64	1.00			4.86	0.69	0.98
EGCG				3.89	0.52	0.99			2.88	0.71	0.98
Cis + EGCG	0/0	1:17.1	0.68	0.08	0.68	0.95	1:1.34	0.71	0.66	0.55	0.99
	0/4		0.41	0.05	0.55	0.98		0.20	0.18	0.43	0.99
	4/0		1.43	0.17	0.63	0.99		0.38	0.36	0.51	0.94
TH5				31.4	1.02	1.00			24.6	1.36	0.99
TH5 + EGCG	0/0	2.6:1	1.38	3.25	1.13	0.96	2:1	0.79	1.16	0.60	0.97
	0/4		0.16	0.04	0.51	0.99		0.56	0.99	0.62	0.97
	4/0		0.22	0.05	0.57	1.00		0.67	0.83	0.53	0.94
TH6				5.52	1.35	1.00			2.99	1.07	1.00
TH6 + EGCG	0/0	0.63:1	2.10	4.53	1.26	1.00	0.66:1	2.17	1.20	0.54	1.00
	0/4		0.45	0.97	0.45	1.00		0.24	0.13	0.22	0.98
	4/0		0.56	1.20	0.53	0.99		0.58	0.32	0.33	1.00
TH7				21.2	0.80	0.98			17.9	0.97	0.96
TH7 + EGCG	0/0	1.97:1	2.47	5.39	1.12	1.00	1.79:1	2.56	1.41	0.57	0.99
	0/4		0.45	0.97	0.45	1.00		0.24	0.13	0.25	0.99
	4/0		0.53	1.16	0.61	1.00		0.36	0.20	0.33	1.00

0/0 h: Both the drugs are added at the same time; 0/4 h: cisplatin or TH5/TH6/TH7 is added first, followed by the addition of EGCG 4 h later; 4/0 h: converse of 0/4 h sequence; CIs: combination indices; ED_{50}: effective dose 50; D_m: median effect dose; m: exponent defining the shape of the dose effect curve; r: linear correlation co-efficient of the median effect plot.

Results

Growth inhibitory effects of single drugs. Table I presents the IC_{50} values and resistance factors (RF) for cisplatin, TH5, TH6, TH7 and EGCG, as applied to the ovarian cancer cell lines. The RF is defined as the ratio of the IC_{50} value of a drug for the resistant cell line to that of the parental cell line. It can be seen that all the three *trans*-palladiums (TH5, TH6, and TH7) and the phytochemical EGCG are less active than cisplatin against the four ovarian cancer cell lines, except that TH6 was found to be slightly more active than cisplatin in both A2780^{cisR} and A2780^{ZD0473R} cell lines. Cisplatin had the highest RFs and TH5 had the lowest. Lower RF values (close to 1 or less) for TH5, TH6, TH7 and EGCG than cisplatin indicate that these compounds are equally or better able to induce cell death of the resistant cell lines compared to the parental.

Growth-inhibitory effects of drugs in combination. Combination of cisplatin, TH5, TH6 and TH7 with EGCG was investigated as a function of the sequence of administration and concentration against A2780 and A2780^{cisR} cell lines, with CIs being used as a measure of combined drug action. The dose-effect parameters in terms

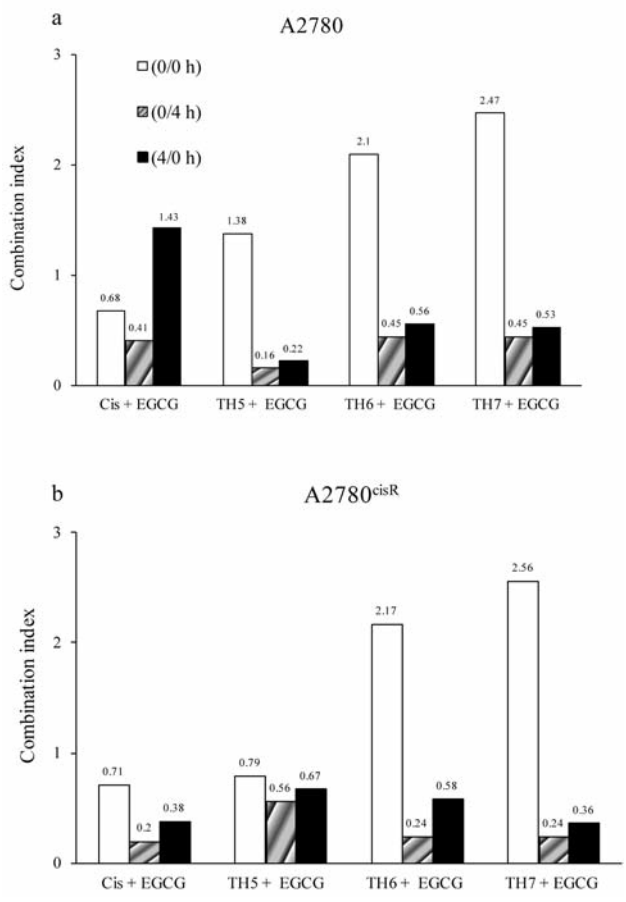

Figure 2. *Combination indices (CIs) applying to the 0/0 h, 0/4 h and 4/0 h combinations of cisplatin (Cis), and trans-palladiums (TH5, TH6 and TH7) at the median effect dose (ED_{50}) with EGCG in A2780 (a) and A2780^{cisR} (b) cell lines, calculated based on the pooled data from three to five individual experiments.*

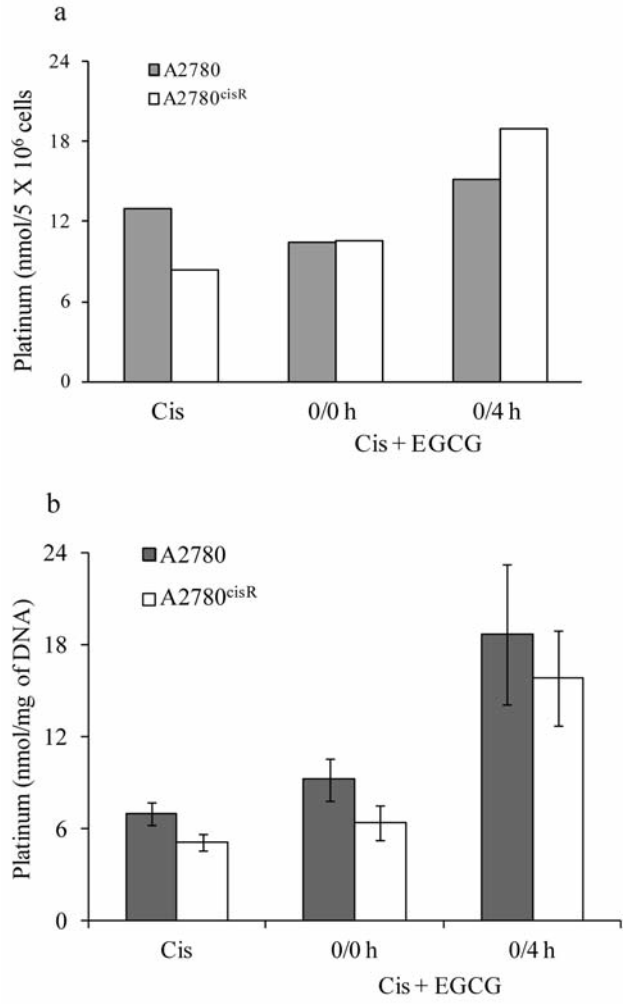

Figure 3. *Total intracellular platinum levels (a) and platinum–DNA binding levels (b) in the A2780 and A2780^{cisR} cell lines treated with cisplatin and its 0/0 h and 0/4 h combination with (−)-epigallocatechin-3-gallate (EGCG); the values are averages of at least three independent experiments.*

of D_m (median-effect dose), m (sigmoidal shape,) and r (conformity as linear correlation co-efficient) are presented in Table II and Figure 2 provides the CI values at the median effect dose (ED_{50}). The results show that co-administration of cisplatin with EGCG (0/0 h) produces only weak synergism in both cisplatin-sensitive and cisplatin-resistant cell lines. In contrast, bolus administration of EGCG with TH5, TH6 or TH7 produces marked antagonism, except for TH5 in the A2780^{cisR} cell line (where a mild synergism is observed). As applied to administration of the drugs with a time gap, both sequences of administration were found to produce high synergism in both A2780 and A2780^{cisR} cell lines in the case of *trans*-palladiums; in the case of cisplatin, whereas the 0/4 h sequence of administration is synergistic, the 4/0 h sequence is antagonistic in the A2780 cell line and synergistic in the A2780^{cisR} cell line.

Cellular accumulation and DNA binding level. As the activity of platinum drugs is believed to be associated with their binding to DNA, the effect of the presence of EGCG on both the cellular accumulation of platinum and the level of platinum–DNA binding in A2780 and A2780^{cisR} cell lines applying to the highly-synergistic 0/4 h and mildly- synergistic 0/0 h combinations was determined (Figure 3). Likewise, the effect of presence of EGCG on the cellular accumulation of palladium and the level of palladium–DNA binding in A2780 and A2780^{cisR} cell lines applying to highly-synergistic 0/4 h and antagonistic 0/0 h combination, was also determined (Figure 4). It was found that for the 0/4 h combination of cisplatin with EGCG in parental A2780 and cisplatin-resistant A2780^{cisR} cell lines, both the cellular accumulation of

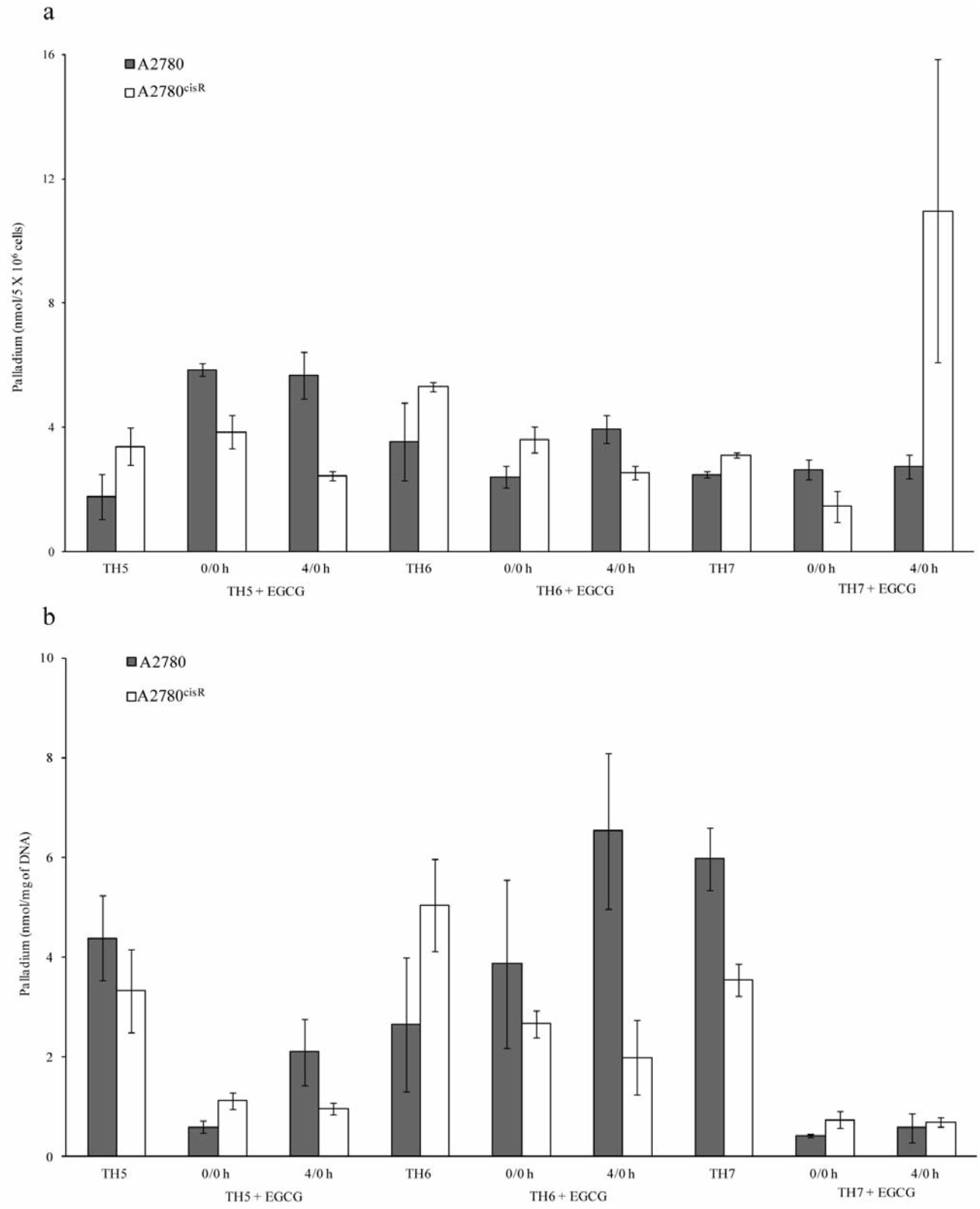

Figure 4. *Total intracellular palladium levels (a) and palladium–DNA binding levels (b) in the A2780 and A2780^{cisR} cell lines treated with trans-palladium compounds (TH5, TH6 and TH7)-alone and their 0/0 h and 4/0 h combination with EGCG; the values are averages of at least three independent experiments.*

platinum and level of platinum–DNA binding are greater than those from the 0/0 h combination and also from that of cisplatin-alone. As applied to TH5-, TH6- and TH7-alone and their 0/0 h and 0/4 h combinations with EGCG, no clear trend can be seen neither for cellular accumulation of palladium nor for the level of palladium–DNA binding. However, the two more active *trans*-palladiums, TH6 and TH7, and their combinations with EGCG were found to be associated with higher palladium–DNA binding levels, especially in the parental A2780 cell line.

Discussion

The lower RFs for *trans*-palladiums (TH5, TH6 and TH7) and EGCG, as compared to cisplatin (close to 1 or less for TH5, TH6, TH7 and EGCG, and 5 or greater for cisplatin), indicate that the compounds are better-able to induce cell kill in resistant cell lines than cisplatin. Trans-planaramine platinum(II) complexes also have much lower RFs than cisplatin, as applied to ovarian cancer cell lines (31, 32). It has been suggested that DNA interstrand crosslinks formed by *trans*-platinum compounds are not generally repaired as efficiently as the intrastrand crosslinks formed by cisplatin (33). Although this explanation may be equally true for *trans*-palladium compounds TH5, TH6 and TH7, the greater lability of palladium–DNA adducts (as compared to platinum–DNA adducts) may serve to complicate the matter in the case of TH5, TH6 and TH7. As applied to apoptosis induced by EGCG, binding with DNA is not relevant (as the cell kill is brought about by a different mechanism) and hence mechanisms of platinum resistance, also, may not be relevant in regard to the drug.

In considering synergism from the combinations of green tea antioxidant EGCG with cisplatin and *trans*-palladiums, it is noted that the polyphenol has a growth-inhibitory effect on a number of different ovarian cancer cell lines (34-36). Although EGCG generally acts as an antioxidant, it may also act as a pro-oxidant at high concentrations and can thus damage cells (37). It has been reported that ovarian cancer cells produce endogenous hydrogen peroxide upon treatment with EGCG that serves to amplify the cytotoxic effect of cisplatin by 3- to 6-fold (38). The high synergism observed for the 0/4 h combination of cisplatin with EGCG is in agreement with reported results (20). It has been suggested that EGCG may accentuate the oxidative stress that would serve to inhibit the growth of ovarian cancer cells and sensitize them to platinum action. The cytotoxic effect of Cis is believed to be closely associated with the increased generation of reactive oxygen species (ROS). The elevation of the level of ROS would serve to reduce that of intracellular thiols (such as glutathione, which is the main intracellular antioxidant in the body) that are involved in detoxification of ROS. As the level of intracellular thiols decreases, less platinum is de-activated, resulting in increased binding of platinum with DNA. It has been suggested that the addition of cisplatin first, 4 h before the addition of EGCG, to the cancer cells would serve to increase the activity of ROS in the cells. In contrast, EGCG is a polyphenolic compound that has been shown to provide protection against DNA damage by acting as a scavenger for free radicals (39) so that in presence of EGCG, more platinum may be deactivated before binding with DNA, thus providing an explanation as to why the 4/0 h combination of cisplatin with EGCG is antagonistic in the A2780 cell line. This may also explain why high synergism is produced from the 0/4 h combinations of TH5, TH6 and TH7 with EGCG. As to the question of why the addition of cisplatin 4 h before the addition of EGCG greatly increases the cellular accumulation of cisplatin in both the A2780 and $A2780^{cisR}$ cell lines, it was suggested that EGCG may also act as a proteasome inhibitor, similarly to bortezomib which retards the cisplatin-induced down-regulation of copper transporter CTR1 that is involved in transporting cisplatin into the cell (40-43).

The multifactorial nature of platinum resistance (which includes reduced uptake, increased efflux, increased de-activation before binding with DNA, increased DNA repair and increased tolerance to platinum–DNA adducts) implies that multiple strategies can be gainfully employed to overcome platinum resistance (at least partially) (44, 45). Although platinum–DNA binding may be a necessary step to induce cell kill, on its own it is not sufficient to induce apoptosis. In actual fact, cell death is brought about by downstream processes in the cell cycle, where many proteins are involved. Platinum resistance is associated with increased expression of transcription factor Nuclear factor kappa-B (NF-κB) and up-regulation of AKT and cyclooxygenase-2 (COX-2) pathways, resulting in cell survival (46-49), whereas EGCG has the converse effect (50-52). Indeed, EGCG is capable of acting on multiple cellular targets and signaling pathways, including induction of cell cycle arrest and apoptosis, modulation of cell signaling by inhibition of NF-κB, mitogen-activated protein kinases (MAPKs), epidermal growth factor receptor (EGFR), and inhibition of metalloproteases, vascular endothelial growth factor (VEGF), proteasome, COX-2 overexpression, and many others (53).

Based on the current findings, about 15% of all solid tumours are driven by NF-κB, as a player, whereas most cancer-preventive agents are believed to be NF-κB inhibitors (54). Aberrant activation of NF-κB can provide protection from apoptosis and stimulate the proliferation of malignant cells, and its overexpression is causally linked to phenotypic changes that are characteristic of neoplastic transformation. Activation of NF-κB occurs in response to a wide variety of stimuli, such as cytokines, growth factors, physiological, physical and oxidative stress, and certain pharmacological drugs and chemicals (4). The convergent step in signal-

induced activation of NF-κB is the phosphorylation of Inhibitory kappa B alpha protein (IκBα) that is done by the enzyme IκB kinase (IKK), leading to its ubiquitination and degradation. Once IκBα is degraded, active NF-κB is translocated into the nucleus. The inhibitory effect of EGCG on NF-κB activation may be due to the inhibition of IKK activity (55, 56). Both cisplatin- and *trans*-platinum-mediated DNA damage has been shown to up-regulate p53 in A2780 ovarian cancer cells (33, 57). It has also been reported that *trans*-palladium complexes, such as *trans*-[1-benzyl-3-tert-butylimidazol-2-ylidene]$_2$PdCl$_2$, can inhibit tumour cell proliferation by arresting cell-cycle progression at the G_2 phase, preventing the mitotic entry of the cell and also causing apoptosis through a p53-dependent pathway (58).

Combinations of cisplatin and three *trans*-palladiums (TH5, TH6 and TH7) with major green tea polyphenol EGCGs were found to produce sequence-dependent synergism, with the 0/0 sequence of administration being least synergistic or even antagonistic, whereas administration of drugs with a 4 h time gap produces greater synergistic outcomes. The results of the study support the idea that sequenced combinations of platinum drugs and tumour-active palladium compounds with selected phytochemicals can provide a means of overcoming drug resistance in ovarian cancer.

Conflicts of Interest

Mohammed Ehsanul Hoque Mazumder, Philip Beale, Charles Chan, Jun Qing Yu and Fazlul Huq declare that they have no financial and personal relationships with other people or organizations that could inappropriately influence their work.

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