Synergism from Combinations of Cisplatin and Oxaliplatin with Quercetin and Thymoquinone in Human Ovarian Tumour Models

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Abstract. *The development of drug resistance remains one of the major hurdles in cancer chemotherapy, particularly so for ovarian cancer. Combination of drugs acting synergistically in combination can offer a means of overcoming drug resistance. In this study, two tumour-active phytochemicals, quercetin and thymoquinone, were combined with two platinum drugs, cisplatin and oxaliplatin, with the aim of providing a means of overcoming drug resistance. Two human epithelial ovarian cancer cell lines, A2780 and its cisplatin-resistant form (A2780^{cisR}) were treated with binary combinations of cisplatin and oxaliplatin with quercetin and thymoquinone using three sequences of administration. Cell viability was quantified using the (MTT) reduction assay. The combined drug action was analysed based on the equations derived by Chou and Talalay (1984). Greatest synergism was observed when the phytochemical was added first followed by platinum drug 2 h later and the least synergism (often additive to antagonistic) was observed when the two compounds were administered as a bolus. It is suggested that the addition of the phytochemical 2 h before platinum drug may sensitize cancer cells to platinum action, thus offering a means of overcoming drug resistance. The results may be highly significant clinically if found to be confirmed in vivo.*

Ovarian cancer is the most leading cause of death from gynaecological cancer in the Western world (1). This may be due to poor prognosis and the absence of early symptoms so that at the time of diagnosis, the disease would have spread beyond the ovaries in approximately two-thirds of patients (2). Platinum drugs such as cisplatin (Cis) and oxaliplatin (Oxa) (Figure 1) are routinely used to treat various types of cancer, including the ovarian cancer. However, both Cis and Oxa suffer from major drawbacks related to their side effects and drug resistance. The main reason for low survival in ovarian cancer is acquired resistance to chemotherapy. Hence, overcoming such drug resistance is bound to have a major impact towards successful treatment for ovarian cancer (3).

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 often exert their antitumour activity through the regulation of cell signalling pathways different from those applying to platinum drugs, it is logical to believe that platinum drugs in combination with the dietary compounds may exert enhanced antitumour activity through synergistic action and/or compensation for the adverse effects. Such combination treatment may also reduce the systemic toxicity caused by chemotherapies or radiotherapies because of lower doses required (4).

Studies have shown that the major factors involved in the development of drug resistance are multidrug resistance gene, nuclear factor-κB (NF-κB), and the serine/threonine protein kinase AKT. Based on 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 (5). Aberrant activation of NF-κB can provide protection against apoptosis and stimulate proliferation of malignant cells, and its over expression is causally linked to phenotypic changes that are characteristic of neoplastic transformation. Aberrant 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

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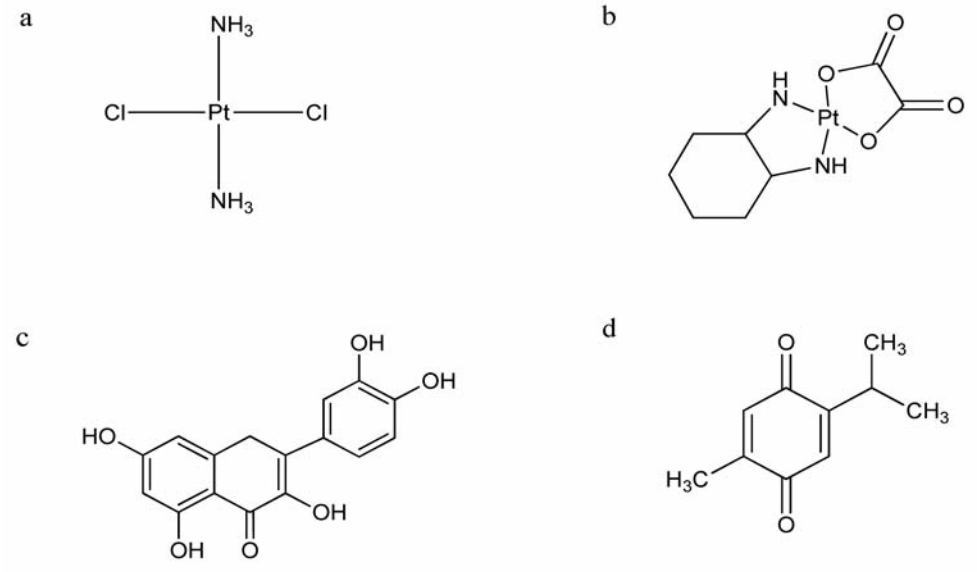

Figure 1. *Structures of a) cisplatin, b) oxaliplatin, c) quercetin and d) thymoquinone.*

chemicals (6). These stimuli target the protein IKB to which is bound the two major subunits of NF-κB, p50 and p65. Phosphorylation and ubiquitination of IKB releases NF-κB, allowing it to translocate into the nucleus as the activated form, where it binds to specific sequences of DNA called response elements (RE). The DNA–NF-κB complex then recruits other proteins that transcribe downstream DNA into mRNA. The mRNA, in turn, is translated into protein that results in a change in cell function. The acquired resistance to Cis in ovarian cancer has been shown to be linked to the activation of NF-κB whereas the chemosensitization of ovarian cancer cells due to the combination of Cis with phytochemicals such as genistein is believed to be due to inactivation of NF-κB (6).

Two common phytochemicals widely distributed in the plant kingdom, quercetin (Quer) and thymoquinone (TQ) (Figure 1), are well-known antioxidants that display a variety of biological activities including chemoprevention and inhibition of tumour growth (7). The structures of Quer and TQ along with those for Cis and Oxa are given in Figure 1. Quer exerts antitumour activity by inhibiting the activation of NF-κB (8). The second phytochemical TQ exerts anti-inflammatory effects and inhibits tumour cell proliferation through modulation of apoptosis signalling, inhibition of angiogenesis, and cell cycle arrest (5). TQ is associated with increased expression of p53 and the downstream p53 target gene, $p21^{WAF1}$. The apoptotic effect of TQ is also modulated by apoptosis B-cell lymphoma (BCL2) protein (9). In addition, it acts as an angiogenesis inhibitor within cells (10). From the above, the combination of platinum drugs such as Cis and Oxa with the phytochemicals Quer and TQ may be supposed to exhibit sequence-dependent synergism. The present study aimed to investigate the effect on the cell kill in the ovarian A2780 cell line and its cisplatin resistant counterpart $A2780^{CisR}$, due to the combination of Cis and Ox with the phytochemicals Quer and TQ. It constitutes a part of our continued study of synergism from combinations of platinum drugs and phytochemicals (11-13).

Materials and Methods

Drugs and cell lines. Cis was prepared according to a previously described method (14). Oxa, Quer and TQ were obtained in the powder form from SIGMA Aldrich Sydney Australia. Oxa, Cis and TQ were initially dissolved in dimethyl formide (DMF) followed by the addition of milliQ (mQ) water (at a ratio 1:5) to give 1 mM stock solution. Quer was dissolved in ethanol to prepare a 1 mM solution. The solutions were filtered using a DISMIC-25cs ADVANTEC filter (Cellulose Acetate, 0.20 μm hydrophilic, pressure limitation: 0.51 MPa) to sterilize. The stock solutions were serially diluted with freshly prepared RPMI-1640 medium to produce a range of final concentrations from 0.0005 to 100 μM. A2780 (parent) and $A2780^{cisR}$ (Cis-resistant type) ovarian cancer cell lines were obtained from Ms. Zhang from the Royal Prince Alfred Hospital, University of Sydney, Australia. The cell lines were sub-cultured in RPMI 1640 medium that was prepared in 10% (FCS), 1 mM Hepes, 5.6% sodium bicarbonate and 200 mM glutamine.

Cytotoxicity assays. The cell kill due to single dugs and drugs in combination was determined using the MTT reduction assay (15). Briefly, 4000 to 6000 cells per well in RPMI-1640 medium were seeded into a flat-bottomed 96-well culture plate 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 (37˚C, 5% carbon dioxide in air, pH 7.4) for 72 h. After preparation of serial fivefold dilutions of the drugs in 10% FCS/RPMI-1640 medium (for Oxa: 0.064-8.0 μM, for Cis and TQ: 0.32-40 μM: and for Quer: 1.6-200 μM), 100 μl of drug were added to equal volumes of cell culture in triplicate wells and incubated 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 (IC_{50}) values using the sequences: 0/0, 0/2 and 2/0, where 0/0 meant that both the drugs were added at the same time, 0/2 meant that Cis or Oxa was added first followed by Quer or TQ 2 h later, and 2/0 meant the converse. The concentration ranges were: Cis: 0.26-4.09 μM and 1.66-26.52 μM; Oxa: 0.16-2.62 μM and 0.59-9.41 μM; Quer: 9.08-145.22 μM and 10.38-166.10 μM; and TQ 2.28-36.49 μM and 1.93-30.83 μM in 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 (15).

The combined action of the drugs was studied using the median effect analysis. The combination index (CI) was calculated based on the pooled data from 3 to 5 individual experiments each comprising at least three data points for each drug alone and for the drug combinations. The combination index (CI) for two drugs in combination can be calculated using the following equation (16, 17).

$$CI = \frac{D_1}{D_{1x}} + \frac{D_2}{D_{2x}}$$

where D_1 and D_2 in the numerator represent the concentrations of compounds 1 and 2 in combination to achieve x% inhibition whereas D_{1x} and D_{2x} represent concentrations of compounds 1 and 2 to achieve x% inhibition when present alone. D_x can be readily calculated from the following equation. In the equation D_x denotes dose of drug, D_m is the median-effect dose ED_{50}, f_a is the fraction of cells affected (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.

$$D_x=D_m[f_a/(1-f_a)]^{1/m}$$

A CI of <1, =1 and >1 indicates respectively synergism, additivity and antagonism respectively, of the combined drug action. The linear correlation coefficient, 'r' indicates the goodness of fit for the pooled data (where r=1 is a perfect fit). For the median effect plot for the cell culture system, r should be greater than 0.95 (16, 17). The CI, D_m and r values were calculated using Calcusyn software (V2) (Biosoft, UK).

Platinum accumulation and platinum–DNA binding. Since activity of platinum drugs is believed to be associated with DNA, most synergistic (2/0) and least synergistic (0/0) combinations of Cis with Quer and TQ were selected for the determination of cellular platinum accumulation and platinum–DNA binding level. 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 and platinum–DNA level was a modification of that described by Di Blasi *et al*. (18). Platinum drugs and phytochemicals 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^{-1}). 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 a centrifuge tube and spun for 2 min at 3500 rpm 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.

Table I. *IC_{50} values of cisplatin, oxaliplatin, quercetin and thymoquinone for the human ovarian cancer A2780 and A2780^{cisR} cell lines.*

Compound	IC_{50} (μM)		RF
	A2780	A2780^{cisR}	
Cisplatin	0.67±0.06	4.14±0.90	6.18
Oxaliplatin	0.41±0.20	1.47±0.44	3.59
Quercetin	22.69±3.86	25.95±5.34	1.14
Thymoquinone	5.70±0.68	4.82±1.56	0.85

Cellular accumulation. Cell pellets from drug combinations were suspended in 0.5 ml 1% Triton-X, held on ice while being sonicated. Total intracellular platinum content was determined by graphite furnace atomic absorption spectroscopy (AAS) using a Varian SpectrAA-240 plus with GTA 120 atomic absorption spectrophotometer (Varain Australia Pty Ltd.) (19).

Platinum–DNA binding. DNA was isolated from cell pellets, using H440050 JETQUICK Blood DNA Spin Kit/50 (Austral Scientific Pty Ltd) according to the modified protocol of Bowtell (20). DNA content was determined by UV spectrophotometry (260 nm) (Varian Cary 1E UV-Visible Spectrometer with Varian Cary Temperature Controller) and the platinum level was determined by graphite furnace. AAS. A_{260}/A_{280} ratios were found to be between 1.75 and 1.8 for all samples, ensuring high sample purity (21), and the DNA concentration was calculated according to the equation:

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

Results

Growth-inhibitory effect of single drugs. Table I gives the IC_{50} values of the compounds for the A2780 and A2780^{cisR} cell lines. The resistance factor (RF) is defined as the ratio of the concentration of the drug required for 50% cell kill in the resistant cell line to that in the parent cell line. Between the two platinum drugs, Oxa was found to be more active than Cis, and in fact it was also more active than all the phytochemicals. Among phytochemicals, TQ was the most active compound, with higher activity in the resistant cell line than in the parent cell line. Although the two phytochemicals were less active than Cis and Oxa, they had RF than the platinum drugs.

Growth-inhibitory effects of drugs in combination. As the main aim of the study was to quantify synergism in activity from the combinations of selected platinum compounds with

Table II. *Dose–effect parameters applying to combinations of Cis and Oxa with Quer and TQ 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	CI at ED_{50}	D_m	m	r
Cis				1.01	0.58	1.00			5.51	1.10	0.99
Oxa				0.24	0.64	0.93			0.54	0.42	0.96
Quer				39.82	1.11	1.00			17.97	1.05	0.99
TQ				12.72	0.66	0.99			4.00	0.59	1.00
Cis + Quer	0/0	1:35.55	0.94	0.61	1.15	1.00	1:6.26	0.40	0.64	0.74	1.00
	0/2		0.88	0.57	0.95	1.00		0.46	0.74	0.75	1.00
	2/0		0.72	0.47	0.78	0.99		0.27	0.43	0.59	0.97
Cis + TQ	0/0	1:8.92	0.81	0.61	0.93	1.00	1:1.16	1.00	1.87	0.83	1.00
	0/2		0.42	0.31	0.73	0.99		0.10	0.18	0.37	0.98
	2/0		0.28	0.21	0.49	1.00		0.03	0.05	0.24	1.00
Oxa + Quer	0/0	1:56.75	0.91	0.15	0.52	0.99	1:17.90	0.50	0.16	0.57	1.00
	0/2		1.12	0.19	0.61	0.99		0.68	0.22	0.61	1.00
	2/0		0.92	0.15	0.57	0.99		0.36	0.12	0.54	0.97
Oxa + TQ	0/0	1:14.25	1.38	0.26	0.81	0.95	1:3.31	1.12	0.42	0.62	0.97
	0/2		0.67	0.13	0.41	0.92		0.08	0.03	0.24	0.93
	2/0		0.86	0.16	0.49	0.96		0.33	0.13	0.42	0.98

CI: combination index; ED_{50}: drug concentration required for 50% cell kill; m: exponent defining the shape of the dose–effect curve; r: goodness of fit for the pooled data.

the phytochemicals in two human ovarian cancer cell lines, activities of the compounds alone and in combination were determined. Table II gives dose–effect parameters in terms of median-effect dose, shape (sigmoidicity), conformity (linear correlation coefficient), represented as D_m, m and r respectively and Figures 2 and 3 show the CIs at the ED_{50} as applied to the human ovarian A2780 and A2780^{cisR} cancer cell lines. The results show that combinations of Cis and Oxa with Quer and TQ produce sequence-dependent synergism detailed as follows. For the combinations of Cis with Quer in the A2780 cell line, only weak synergism was observed for all three sequences of administration, with the bolus addition being least synergistic and the 2/0 sequence of administration being the most synergistic. In the A2780^{cisR} cell line, much greater synergism was observed for all the three sequences of administration of Cis and Quer, with the greatest synergism being observed again for the 2/0 sequence. For the combinations of Cis with TQ, the bolus addition was found to be least synergistic (additive or almost additive), whereas the 2/0 sequence of administration was the most synergistic. The degree of synergism was found to be very high for the 2/0 sequence of administration of Cis and TQ. For the combinations of Oxa with Quer in the A2780 cell line, all three sequences of administration were close to being additive, with the 0/2 sequence being slightly antagonistic. In the A2780^{cisR} cell line, all three sequences of administration of Oxa and Quer were found to be synergistic, with the greatest synergism being produced from the 2/0 sequence and the least from the 0/2 sequence. In the combinations of Oxa with TQ, the 0/2 addition was found to be most synergistic in the parent A2780 cell line, as it was also in the resistant A2780^{cisR} cell line.

Cellular platinum accumulation and platinum–DNA binding level. Figure 4 shows the cellular accumulation of platinum and platinum–DNA binding levels in A2780 and A2780^{cisR} cell lines applying to the 0/0 h and 2/0 h combinations of Cis with Quer and TQ. Figure 5 provides the same for the 0/0 h and 2/0 h combinations of Oxa with Quer and TQ. It was found that for the combinations of Cis with Quer and TQ in the parent A2780 cell line, the cellular accumulation of platinum resulting from the 2/0 combination was significantly greater than that resulting from the 0/0 combination and also that from the equivalent concentration of Cis alone. Generally platinum accumulations in the A2780 cell line were higher than those in the A2780^{cisR} cell line (except for the 0/0 h and 2/0 h combinations of Cis with Quer, where it was found to be greater in the resistant A2780^{cisR} cell line). The intracellular platinum level was highest from the 2/0 h combination of: Cis with TQ in the A2780 cell line and Cis and Quer in the A2780^{cisR} cell line. The same combinations of Cis and Quer also resulted in the highest platinum–DNA binding levels. For the combinations of Oxa with Quer and TQ in A2780 and A2780^{cisR} cell lines, both the cellular accumulation of platinum and platinum–DNA binding levels resulting from all the combinations were found to be significantly greater than those resulting from the equivalent concentration of Oxa

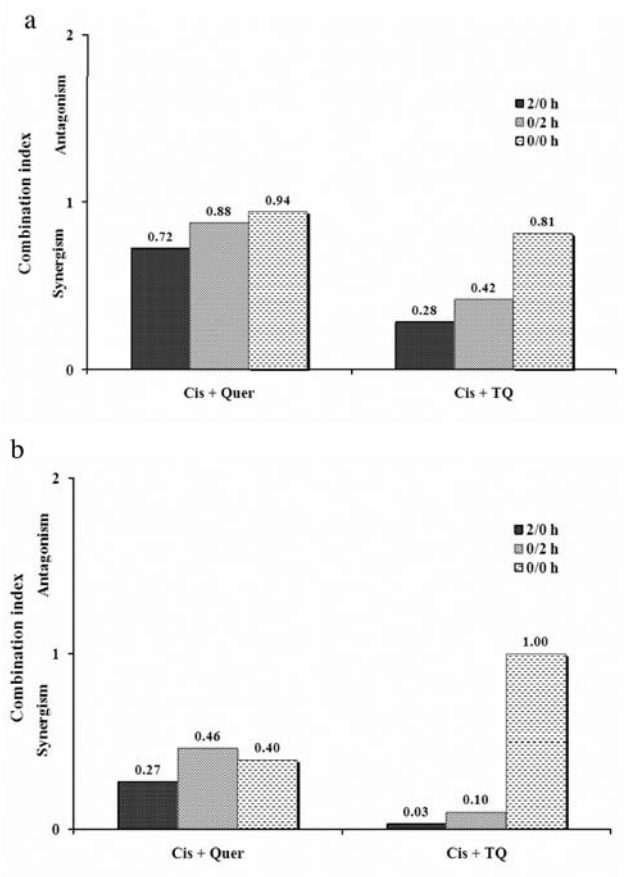

Figure 2. *CIs applying to combinations of Cis with phytochemicals (Cis-Quer and Cis-TQ) at the Median Effect Dose (ED_{50}) in: (a) A2780 cell line, and (b) A2780^{cisR} cell line.*

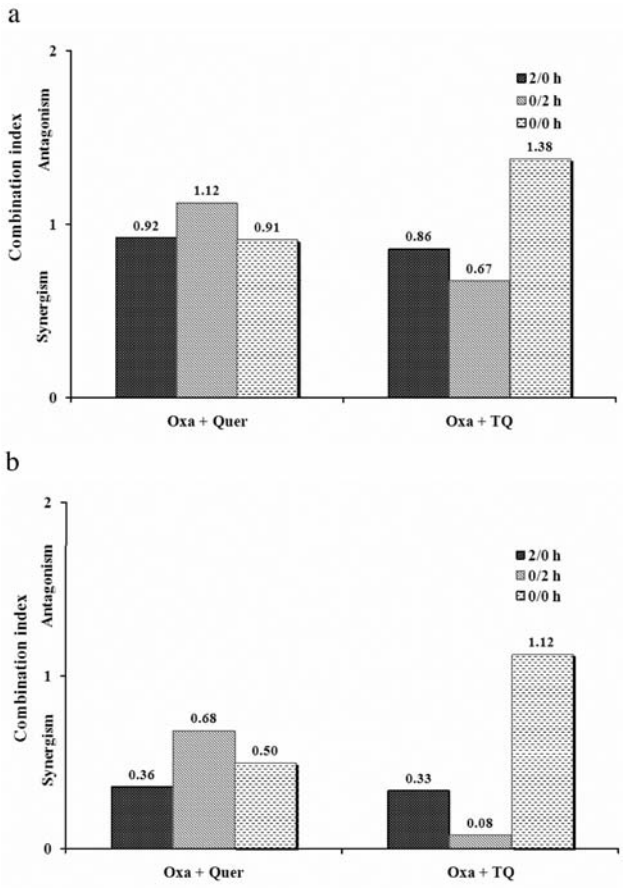

Figure 3. *CIs applying to combinations of Oxa with phytochemicals (Oxa-Quer and Oxa-TQ) at Median Effect Dose (ED_{50}) in: (a) A2780 cell line, and (b) A2780^{cisR} cell line.*

alone and the values in the resistant A2780^{cisR} cell line were higher than those in the parent A2780 cell line (except for the combination of Oxa with TQ where the converse was found to be true). However, uncertainties in measurement make this difference not significant.

Discussion

In this study, we investigated the sequence–dependence of combined drug action for the binary combinations of platinum drugs Cis and Oxa with the phytochemicals Quer and TQ. It was found that the administration of the phytochemical 2 h before that of the platinum drug produced synergistic outcomes whereas the bolus addition was generally close to being additive or antagonistic. The degree of synergism of the 2/0 combination was generally found to be greater in the resistant A2780^{cisR} cell line than in the parent A2780 cell line. Much greater synergism resulting from the 2/0 combination of the platinum drugs with the phytochemicals indicates that the incubation of the cancer cells with the phytochemical for a short period before the addition of the platinum drug served to stimulate the ovarian cancer cells for the cell killing effect due to the platinum drug. This enhanced cell kill cannot be attributed simply to the antioxidant role played by the phytochemicals. When the phytochemicals act as antioxidants, they would serve to reduce oxidative stress, resulting in sparing of cellular antioxidants such as glutathione. Increased glutathione concentration would cause increased deactivation of platinum before its binding with the DNA. However, the increased platinum–DNA binding level observed with the 2/0 sequence of administration clearly indicates that the above explanation is not true, and in any case might be too simplistic. Perhaps the results point to the complexity of the situation due to the involvement of multiple pathways associated with drug resistance as well as with apoptosis. A further point to note is that whereas the cell–killing effect due to single drugs or combination of drugs was measured after a period of 72 h of

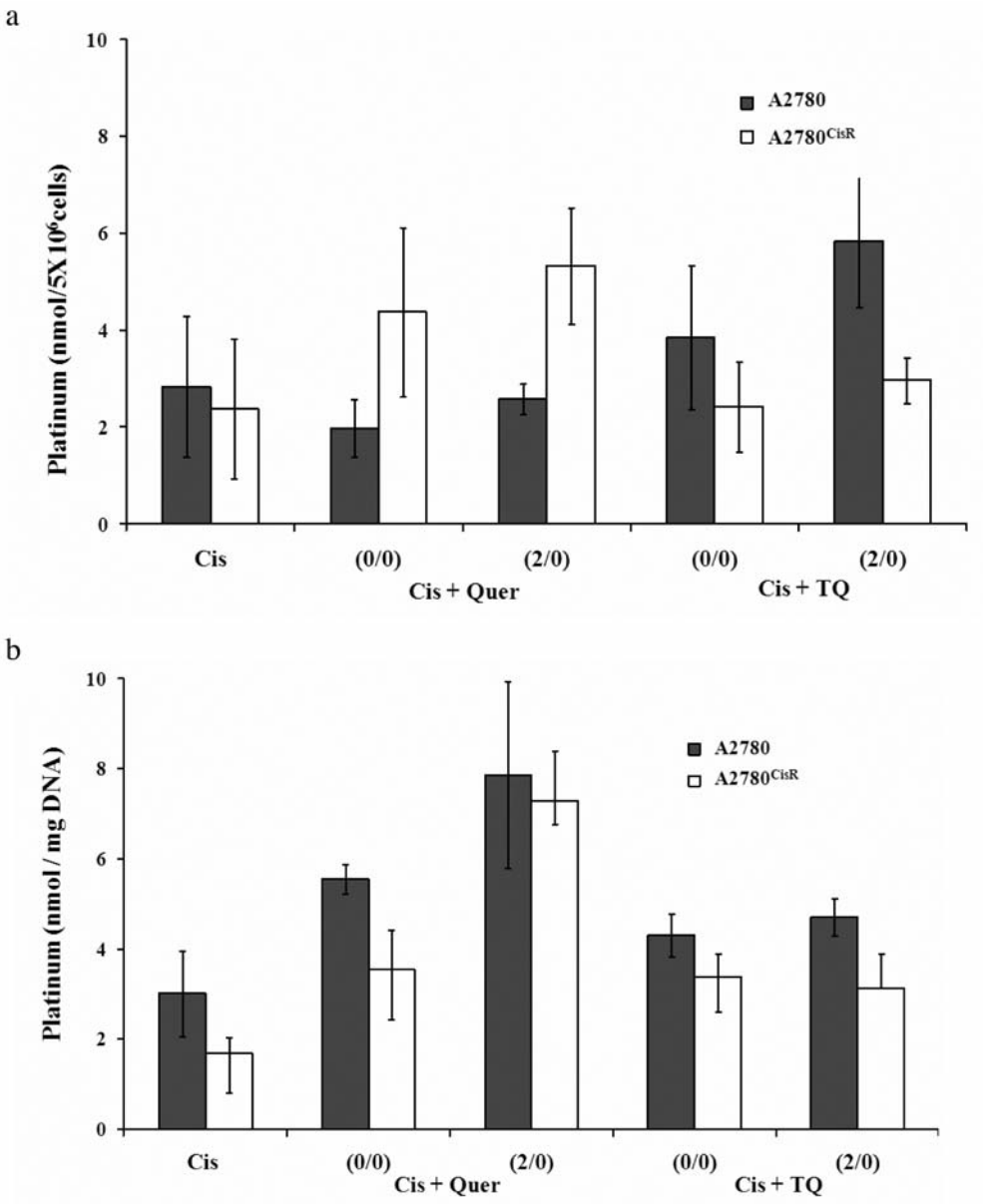

Figure 4. *(a) Total intracellular platinum levels from Cis and its combination with Quer and TQ in the A2780 and A2780^{cisR} cell lines, (b) platinum–DNA binding levels in A2780 and A2780^{cisR} cell lines from Cis and its 0/0 h and 2/0 h combination with Quer and TQ.*

incubation, the cellular accumulation of platinum and the platinum–DNA binding level were measured after 24 h of incubation, thus raising some uncertainty in relating cytotoxicity to the measured platinum accumulation and platinum–DNA binding level. As applied to the intrinsic pathway, although the cell kill by platinum drugs such as Cis and Oxa is believed to be initiated with drug–DNA binding, the apoptosis is actually brought about by downstream processes in the cell cycle in which many different proteins are bound to play key roles. The situation is further complicated by the fact that platinum drugs such as Cis and Oxa (like other many other anticancer drugs) can also bring about apoptosis by an extrinsic pathway in addition to the intrinsic one. The intrinsic pathway is triggered by the p53 tumour–suppressor in response to DNA damage and other types of severe cell stress, ultimately resulting in the activation of caspases 3, 6, and 7, which are responsible for inducing apoptosis. In contrast, the extrinsic pathway stimulates apoptosis independently of p53. Ligand-induced activation of (DR4) and (DR5) leads to the rapid assembly

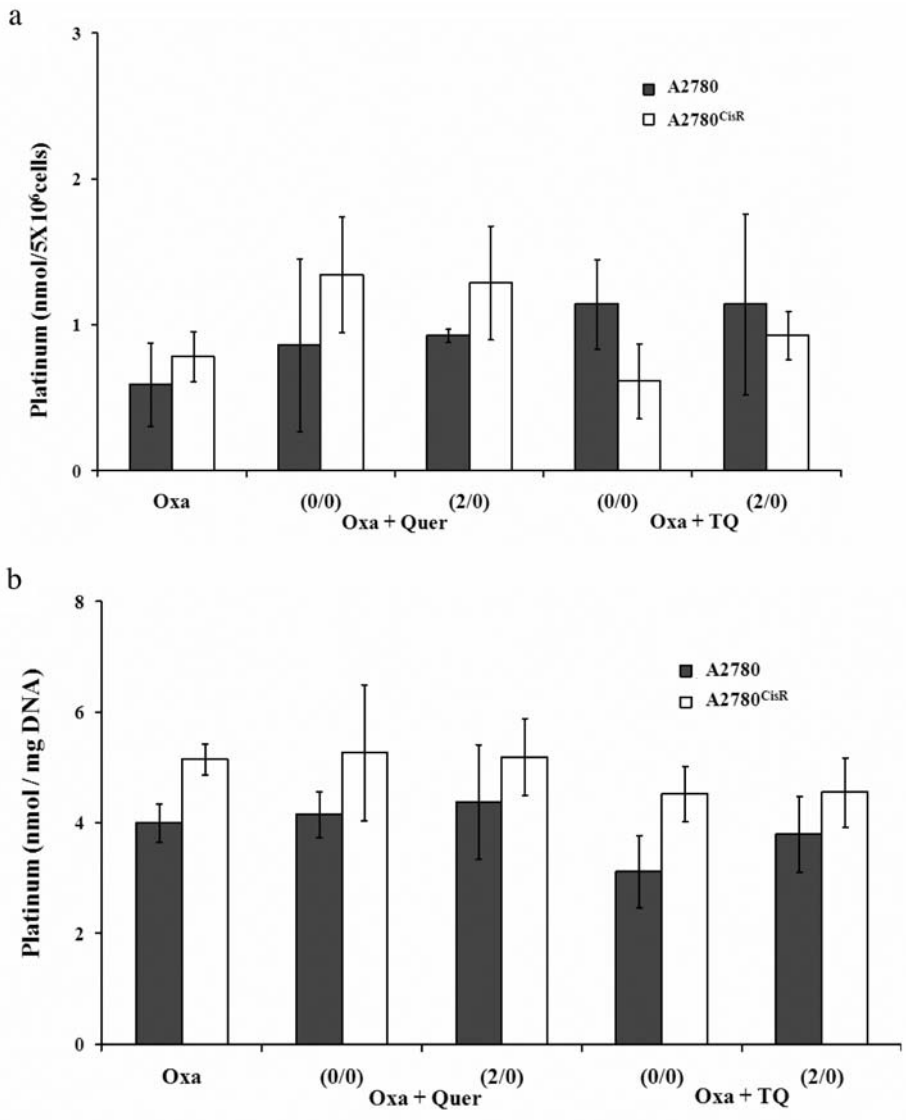

Figure 5. *(a) Total intracellular platinum levels from Oxa and its combination with Quer and TQ in the A2780 and A2780^{cisR} cell lines, (b) platinum–DNA binding levels in A2780 and A2780^{cisR} cell lines from Oxa and its 0/0 h and 2/0 h combination with Quer and TQ.*

of the death-inducing signalling complex (DISC) and the recruitment of initiator caspases 8 and 10 through the adaptor Fas-associated death domain (FADD).

Recently, it has been reported that greater cell kill was produced from Cis alone when it was administered in two aliquots with a time gap than as a bolus. The higher cell kill was also reported to be associated with higher levels of intracellular platinum accumulation and platinum–DNA binding. It was hypothesized that the administration of the first aliquot of Cis placed cancer cells under oxidative stress created by depletion of cellular thiols due to their binding with Cis. When the second aliquot of Cis was added, more of it bound with DNA, thus resulting in increased apoptosis. The authors further suggested that different apoptotic pathways of Cis might become more significant at different time points so that the sequenced administration of Cis in two aliquots could be looked upon as being the combination of two drugs with distinctly different mechanisms of action (22).

In spite of the complexity of the situation described above, both platinum resistance and the enhancement of platinum action due to its combination with the phytochemicals appears to be related to the multidrug resistance gene NF-κB. Whereas the resistance to platinum drugs is associated with aberrant activation of NF-κB, all the two selected phytochemicals are known to dampen its expression. Although only about 15% of all solid tumours are driven by

NF-κB as a player, most cancer preventive agents are believed to be NF-κB inhibitors (5). We suggest that prior incubation of ovarian cancer cells with Quer and TQ serves to reduce the expression of NF-κB and hence to lower the resistance to platinum drug.

When platinum–DNA binding levels in the parent A2780 and the resistant A2780^{cisR} cell lines resulting from Cis alone and its combinations with Quer and TQ are compared, it is found that the levels are significantly greater in the resistant cell line for all sequences of administration so that whereas the ratio of platinum–DNA binding level in the parent cell line to that in the resistant cell line was close to 3 from Cis alone, the value was less than 1.4 for all combinations of Cis with the phytochemicals. The closeness of the platinum–DNA binding levels in the parent and resistant cell lines, especially for the synergistic combinations of Cis with Quer and TQ, can be seen to indicate that prior incubation of the ovarian cancer cells with the phytochemicals has served to dampen the involvement of DNA repair remaining as a mechanism of platinum resistance.

The greater accumulation of platinum from the most synergistic 2/0 sequence of administration than the least synergistic 0/0 combination (especially so for the parent cell line) can be seen to indicate that the prior incubation of the cancer cells with the phytochemicals has served to stimulate them for increased uptake and/or reduced efflux. Much wider variation in the intracellular platinum accumulations between the parent and the resistant cell lines, especially for the most synergistic 2/0 combination than the least synergistic 0/0 combination, may be indicative of the residual resistance; or it may indicate that this variation may no longer be a critical determinant of resistance for the synergistic combinations. Alternatively, the results may indicate that profound changes in cell kill with the change in the sequence of administration are more attributable to the changes in the downstream processes *e.g.* changes in the expression of NF-κB.

As to the question why the bolus addition of platinum drugs and the selected phytochemicals was found to be additive to antagonistic in action, it appears that concurrent administration of the two compounds failed to sensitize the ovarian cancer cells to platinum action, perhaps due to less significant reduction in the expression of NF-κB. Thus, the prior incubation of ovarian cancer cells with Quer and TQ appears to be the critical determinant in lowering the expression of NF-κB. It should however be noted that the idea of sensitization of ovarian cancer cells to platinum action due to their prior incubation may not be applicable to all phytochemicals. Because it has been recently reported that addition of Cis 4 h before the administration of curcumin and the green tea antioxidant epigallocatechin-3-gallate produced greater synergistic outcomes than the converse in both the A2780 and A2780^{cisR} cell lines. The cellular accumulation of platinum and platinum–DNA binding resulting from the 0/4 h combinations were also found to be greater as compared to the values from Cis alone, thus providing an explanation for the synergistic action (11).

The results of the present study may have profound implications in therapy, if they are confirmed *in vivo*. However, the study has left many questions unanswered regarding the exact mechanism of the combined drug action. It is believed that proteomic studies designed to probe changes in the expression of key proteins associated with drug resistance may provide further insight. Finally, given its role in angiogenesis, cell invasion, oncogenesis, proliferation and suppression of apoptosis, it may be true to say that NF-κB will continue to occupy a central focus of therapeutic intervention in many diseases including cancer. It is possible that phytochemicals may provide a pool of NF-κB inhibitors.

Conflict of Interest

Meher Un Nessa, 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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