

Synergism in Concomitant Chemoradiotherapy of Cisplatin and Oxaliplatin and their Liposomal Formulation in the Human Colorectal Cancer HCT116 Model

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Abstract. *Background:* We choose to test the effect of associating chemo-radiotherapy at 8 h (the highest level of DNA-platinum) and 48 h (the lower level of DNA-platinum) to clarify if irradiation at the maximum DNA-platinum concentration could improve the synergism. *Materials and Methods:* Growth inhibition of the human colorectal cancer cell line HCT116 treated with cisplatin, oxaliplatin, Lipoplatin™ and Lipoxal™ plus gamma-radiation was determined by a colony formation assay. The synergism was evaluated using the combination index method. *Results:* For 8 h and 48 h exposure to cisplatin or Lipoplatin™, followed by irradiation, drug concentrations higher than IC₅₀ were found to be synergistic, while a lower than IC₅₀ concentration was antagonistic. For oxaliplatin, exposure to a concentration above IC₅₀ for 8 h was synergistic, while the exposure to oxaliplatin (at any concentrations) for 48 h was antagonistic. Lipoxal™ significantly improved synergism compared to its parent drugs. All tested platinum drugs sensitize radiation-treated HCT116 cells by inducing G₂ phase. *Conclusion:* The difference of drug concentrations and the time interval between drug administration and radiotherapy could give different results in chemoradiation therapy.

The addition of chemotherapy to radiotherapy is expected to improve treatment outcome of numerous malignant diseases. This is evident in head and neck, lung, anal and rectal cancers (1-3), but for some other cancers the benefit of such association is less clear (ex. pancreatic cancer) (4). Some

chemotherapeutic agents (as well as other drugs) have also been proposed to enhance the efficacy of radiotherapy, in addition to their potential anti-neoplastic effect.

In vitro, *in vivo* and clinical studies revealed the complexity of the interactions and showed promising results when platinum chemotherapeutic agents were combined with radiation (5, 6). Platinum salts, cisplatin and oxaliplatin have been shown to possess radiosensitizing properties (7-9) by means of increasing apoptotic cell death. In addition, the integration of platinum derivatives into DNA, in close proximity to a radiation-induced SSB, can act synergistically to make the defect significantly more difficult to repair (10). Cisplatin and oxaliplatin appear to be a particularly plausible choice for combination with radiation, since they are not considered to be radio-mimetic drugs (11).

However, the mechanism underlying such radiosensitisation of platinum drugs is not fully understood in most cases. Moreover, the effect of the relative time sequence of the combined treatment of chemotherapy and radiation has not been systematically studied. We recently observed the relationship between exposure time to various concentrations of different platinum salts and the platinum level in different intracellular compartments (cytoplasmic, nuclear and DNA-bound platinum) of HCT116 cells as a function of incubation time (12). Pre-clinical investigation on the concomitant interaction of platinum and radiation at a specific time point might shed some light on the clinical relevance of the optimal synergism and the reduction of systemic toxic effect for colorectal cancer treatment.

Despite the wide range of applications of cisplatin and oxaliplatin, anticancer agents, severe side effects have been reported such as neurotoxicity, nephrotoxicity, ototoxicity and retinopathy (13). In addition, colorectal cancers have been reported to have intrinsic resistance to cisplatin and chronic resistance to oxaliplatin (14, 15), which has been observed in the reduction of the intracellular accumulation of free platinum (16). These severe side-effects and cancer cell resistance to cisplatin and oxaliplatin provoke

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a limitation of their therapeutic efficiency in colorectal cancer treatment. In order to enhance its efficacy and reduce systemic toxicity, the drug is encapsulated in liposomal formulation

Lipoplatin™ and Lipoxal™, the liposomal formulation of cisplatin and oxaliplatin, have been designed with the aim of reducing systemic toxicity, while simultaneously improving the circulation half-life time of the encapsulated drug that is slowly released as liposomes degrade (17, 18). In addition, the liposomal formulation has been suggested to bypass the uptake and efflux transporters, and potentially increase the delivery of free-platinum drugs to the tumor cells. Liposomal drugs have a better tolerance profile and are highly accumulated in the tumor, properties that promise an optimal radiosensitization. A Phase I/II study in advanced gastric cancer showed that Lipoplatin™ radio-chemotherapy is feasible, with minor hematological and non-hematological toxicities (19).

In the current work, we describe the effect of radiotherapy at different times of exposure to these platinum salts (which implies different concentrations of platinum drugs). Another purpose was to compare the synergistic effects between free-platinum formulation, cisplatin and oxaliplatin, with their liposomal formulation, Lipoplatin™ and Lipoxal™. The interaction of platinum and radiation was investigated with respect to colonies formation, combination index and apoptosis. From these data, we assess the impact of these timings on the synergism between chemo-radiotherapies.

Materials and Methods

Cell lines and drugs. The HCT116 colorectal carcinoma cell line obtained from American Type Culture Collection is p53-wild type. Cells were routinely cultured in modified Eagle's medium (MEM) (Sigma, Oakville, Canada) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 1 mM Sodium-Pyruvate, 100 units/ml penicillin and 100 μM streptomycin in a fully humidified incubator at 37°C in an atmosphere containing 5% CO₂. Cisplatin and oxaliplatin were purchased from Sigma-Aldrich. Lipoplatin™ and Lipoxal™, the liposomal formulation of cisplatin and oxaliplatin, respectively, were generously provided from Regulon Inc. (Athens, Greece). All drugs were diluted to the given concentrations in culture medium immediately before use in FBS-free MEM.

Clonogenic assay. The cytotoxicity of platinum and ionizing radiation against HCT116 cell line was assessed by a colony formation assay. For chemoradiation combination therapeutic studies on HCT116 cells, we performed with three groups of treatment: 1) the control group treated with four representative platinum-based drugs only at five concentrations, 2) the group treated with ionizing radiation at four doses of radiation, 3) the group treated with a combination of drugs and radiation, at a constant of the IC₅₀ and LD₅₀ values (IC₅₀ referred to, and LD₅₀ referred to the dose of radiation causing a 50% reduction of cell growth compared with untreated cells). All the experiments began by growing the 1000 cells from a single-cell suspension in 100-mm

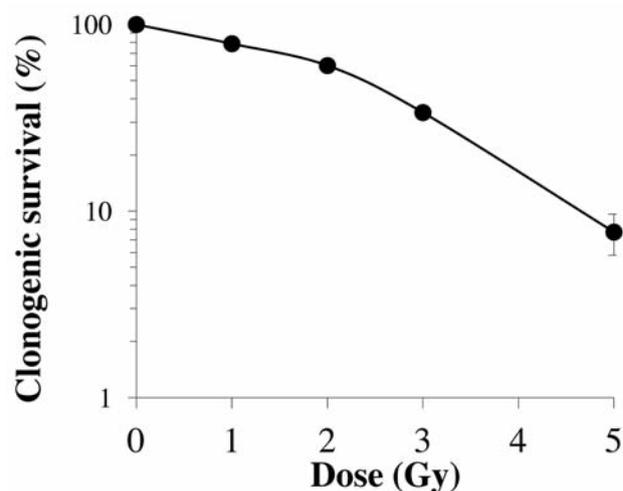


Figure 1. Dose-response curve for radiotherapy-alone on HCT116 cells. Cells were irradiated with gamma-rays (γ -rays) during exponential cell growth using a ⁶⁰Co unit at a dose rate of 1.64 Gy/min. Each point represents the mean of 3 independent experiments and the bars indicate the standard deviation (S.D).

cell culture dishes containing 10 ml of culture medium and incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 24 h. After the culture medium was removed, the cells were washed twice with PBS before following treatments.

For chemotherapy-alone, cells were treated with platinum compounds at appropriate concentrations and further incubated for the desired period. Thereafter, the drugs were removed and the cells were incubated for another 7 days to allow the formation of colonies. Cells were fixed and stained with 0.1% crystal violet, after washing with tap water. Colonies containing more than 50 cells were counted manually to calculate the clonogenic survival fractions. Each experiment was performed at least of 3 times using triplicate cultures for each drug concentration. In addition, the cytotoxicity of platinum drugs alone was examined in order to determine the IC₅₀ values, *i.e.* the concentrations of drugs causing a 50% reduction of cell growth compared with untreated cells.

For radiation treatment, cells were irradiated with gamma-rays (γ -rays) during exponential cell growth using a ⁶⁰Co unit at a dose rate of 1.64 Gy/min. Cells were maintained in MEM supplemented with 10% FBS during all the radiation exposures, which were performed at room temperature. Dose response curves were established for the HCT116 cells using a total with 4 single doses of 1, 2, 3 and 5 Gy.

For concomitant chemoradiotherapy, cells were treated with the platinum compound at appropriate concentrations, which were the same concentrations as the ones used to study the cytotoxicity of chemotherapy-alone. Thereafter, the drug was removed and the cells were further incubated for the desired periods. We selected exposure time of 8 and 48 h as they were previously shown to correlate with the highest and lowest levels of DNA-platinum adducts, concentration in the nucleus, respectively (12). Then, the cells were irradiated with γ -rays at the LD₅₀ value (*i.e.*, at a single dose of 2.3 Gy, obtained from the results of Figure 1). This figure shows the cell survival fraction decreased, as a function of radiation dose. The

2.3 Gy value was obtained on platinum-free HCT116 cells and corresponds to the radiation dose which resulted in a reduction of 50% of the colonies formation.

After treatment with either radiation-alone or concomitant chemoradiotherapy, the cells were incubated for an additional 7 days (to allow the formation of colonies) before cells fixation and counting colonies formation were performed as described above. Each experiment was performed at least 3 times using triplicate cultures for each drug concentration.

The result of colonies formation assays from different group (chemotherapy-alone, radiation-alone and combined treatments) were used to calculate the combination index (CI) in order to assess the levels of synergy, as described in the following section.

Determination of combination index: Antagonism/Synergism/Additivity. Evaluation of the nature of activity of platinum complexes in combination with radiation is mutually non-exclusive modalities, since the plots for drug and radiation and their combination are not parallel to each other. This evaluation and the CI calculation with respect to cell survival were performed according to the method described by Chou and Talalay (20). The CI was evaluated for each drug at 8 and 48 h of incubation. We also sought to determine whether the synergism would be reached at a platinum drug dose equivalent, lower and higher than that of respective IC₅₀. A lower concentration was defined as the average of minimum tested concentration to the concentration at IC₅₀ values, while a higher concentrations was defined as the average of concentration at IC₅₀ values to the maximum tested concentration.

Mathematical evaluation of the data was carried out by computerized analysis of the median dose effect (IC₅₀) and the combination index as well as linear correlation coefficient (r). According to the CALCUSYN software of BIOSOFT (Ferguson, MO, USA) developed by Chou (21), the CI is expressed by the relationship where each term represents,

$$CI = \frac{(Pr)}{(Ptx)} + \frac{(IR)}{(IRx)} + \frac{(Pr)(IR)}{(Ptx)(IRx)}$$

the denominator, (Ptx) is for the concentration of platinum compound "alone" that inhibits colonies formation at x%, and (IRx) is for the dose of radiation-alone that inhibits colonies formation at the same x%. In the numerator, (Pr) + (IR) "in combination" also inhibit the colonies formation also at the same x%. CI values <1.0, and =1.0, and >1.0 indicate synergistic, additive, and antagonistic effects, respectively.

Cell cycle and apoptosis analysis. Cells were treated with platinum drugs at concentrations corresponding to their specific IC₅₀, as determined by the clonogenic assay. After 8 h and 48 h of platinum drug exposure with or without radiation treatment, both adherent and floating cells were washed and allowed to grow for an additional 48 h in culture medium. Cells were harvested by trypsinization, pooled with the culture medium that contained floating cells, and collected by centrifugation (3 min, 1300 rpm). The cell pellets were resuspended in 0.5 ml of PBS and were fixed with 1.5 ml of ice-cold 95% ethanol. Before analysis, 100 µl of FBS were added. The cell pellets were re-suspended in 2 ml PBS and were collected by centrifugation (3min, 1300 rpm). The 200 µl of

staining solution containing 0.5 mg/ml of RNase A and 5 µg/ml of propidium iodide were added, and the suspension was incubated at 37°C for 30 min. The samples were analyzed using FACScan (Becton Dickinson). The cells in the subdiploid (sub-G₁/G₀) region of the histogram are classified as death cells in apoptosis assay. For each sample 10,000 events were analyzed. These analyses were performed on at least three separate cultures.

Statistical analysis. Results were expressed as the mean±SD of three experiments performed in triplicate. *p*<0.05 was considered statistically significant (two-tailed paired Student's *t*-test).

Results

Clonogenic assay for platinum-drugs chemotherapy. The survival of HCT116 clonogenic cells as a function of platinum-based drug concentration for four tested drugs on cells at 8 and 48 h incubation are shown in Figures 2 and 3, respectively, for the four tested drugs. The IC₅₀ values for all tested drugs in HCT116 cells for 8 and 48 h are summarized in Figure 4. After 8 h of incubation with cisplatin, oxaliplatin, Lipoplatin™ and Lipoxal™, the IC₅₀ values were observed at 4.65 µM, 2.29 µM, 41.62 µM and 5.61 µM, respectively. These IC₅₀ values of the four tested drugs were reduced at about 6.4-, 35.8-, 9.95- and 13.7-times of magnitude, respectively, when the incubation time was increased to 48 h.

Clonogenic assay for radiation treatment. According to the clonogenic assay, the cell survival fraction decreased as a function of radiation dose (Figure 1). The LD₅₀ for the irradiated HCT116 cells was 2.30±0.98 Gy. This value was further used in combination with platinum drugs at different concentrations to measure the CI.

Clonogenic assay for chemoradiation combination. Similar plots of clonogenic cell survival were constructed for all chemoradiation combinations. The results are shown in the same graph as those of the platinum-drug treatment-alone at 8 h and 48 h incubation in Figures 2 and 3. The IC₅₀ values for all tested drugs combinations with radiation at LD₅₀ doses in HCT116 cells for 8 and 48 h, are summarized in Figure 4. The platinum concentration needed to obtain the IC₅₀ was considerably reduced when the tested drugs were combined with radiation. Theses IC₅₀ values were reduced at about 11.4-, 7.3-, 44.2- and 10.89-times of magnitude for combination of radiation and cisplatin, oxaliplatin, Lipoplatin™ and Lipoxal™, respectively, when the time of incubation was increased to 48 h.

Concomitance effect of platinum compounds with radiation. The effects of adding radiation to cells treated at platinum drug concentration equivalent to, lower or higher than their respective IC₅₀, in the time interval of 8 h and 48 h platinum drug incubation, are shown in Figure 5A and 5B.

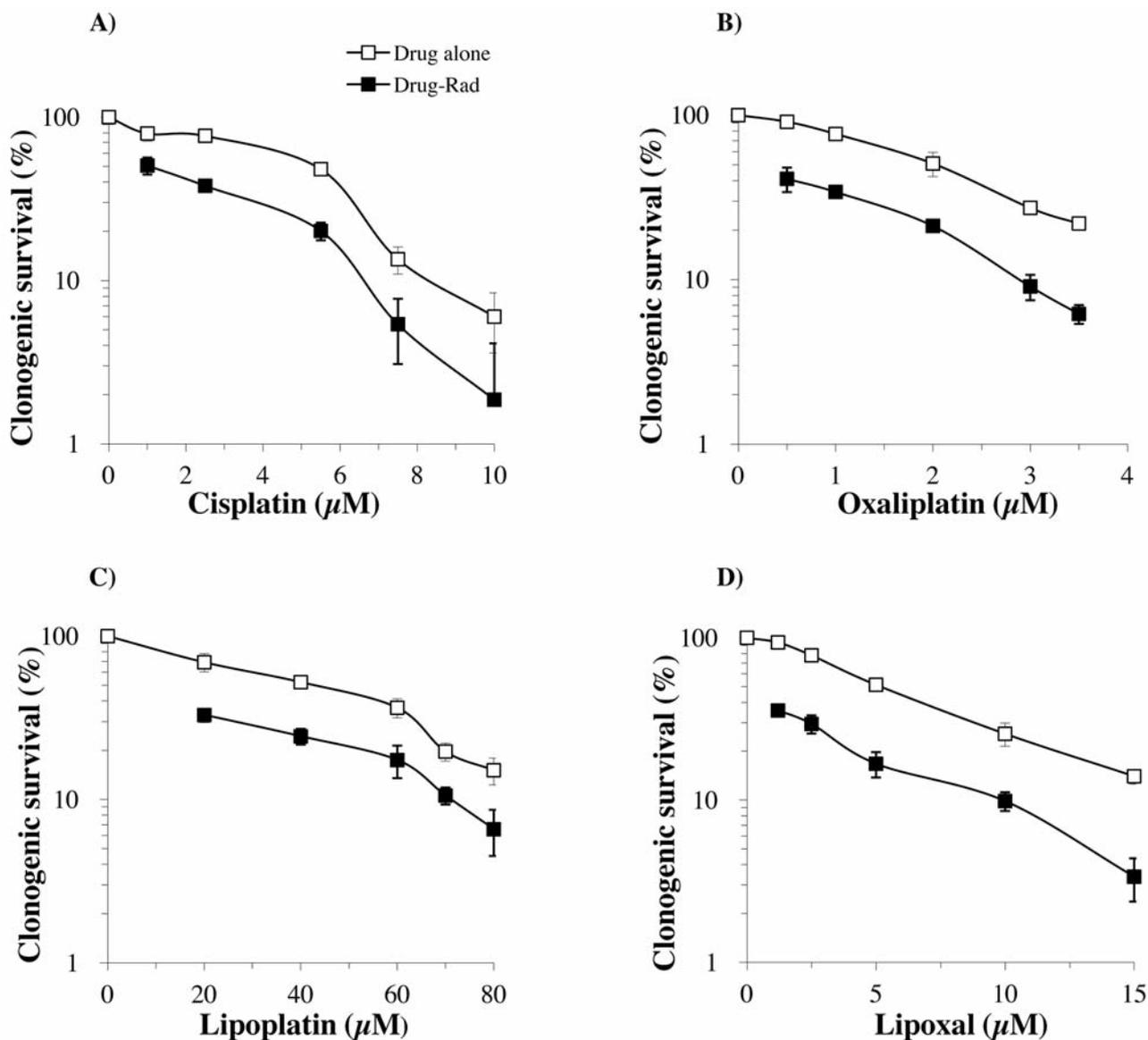


Figure 2. Response curves for chemoradiotherapy on HCT116 cells at 8 h of incubation time. (□) Cells were exposed to various concentrations of each platinum derivative-alone, or (■) in combination with gamma radiation (2.3 Gy). The concentration of the drugs is based on the IC_{50} values, which were previously obtained from the clonogenic assay at 8 h of incubation. Each point represents the mean of 3 independent experiments and the bars indicate the S.D.

Cisplatin: With a similar trend after exposure for 8 h or 48 h, the treatment with cisplatin at IC_{50} showed only an additive effect (CI=1.02 and 1.05). With a dose lower than IC_{50} , the combination with radiation showed an antagonistic (less than additive) effect (CI=1.22 and 1.22), while a synergism was only observed with a concentration above IC_{50} (CI=0.74 and 0.70).

Oxaliplatin: 8 h exposure to oxaliplatin at or below IC_{50} showed only an additive effect (CI=0.99 and 1.05), but a synergism was noted when a dose above IC_{50} was used (CI=0.87). Exposure to oxaliplatin for 48 h (at, above or

below IC_{50}) resulted in an antagonistic effect (CI=1.22, 1.14 and 1.4, respectively).

Lipoplatin: The treatment with Lipoplatin at IC_{50} , for 8 h or 48 h, showed only an additive effect (CI=0.98 and 0.94). Exposure to a concentration below IC_{50} for 8 h showed an additive effect (CI=1.06), while a similar concentration for 48 h exposure resulted in an antagonistic effect (CI=1.19). Treatment with a concentration higher than IC_{50} resulted in a synergism after 8 h or 48 h of exposure (CI=0.77 and 0.77).

Lipoxal: The treatment with Lipoxal at, above or below the IC_{50} value for 8 h, showed a synergism (CI=0.88, 0.89

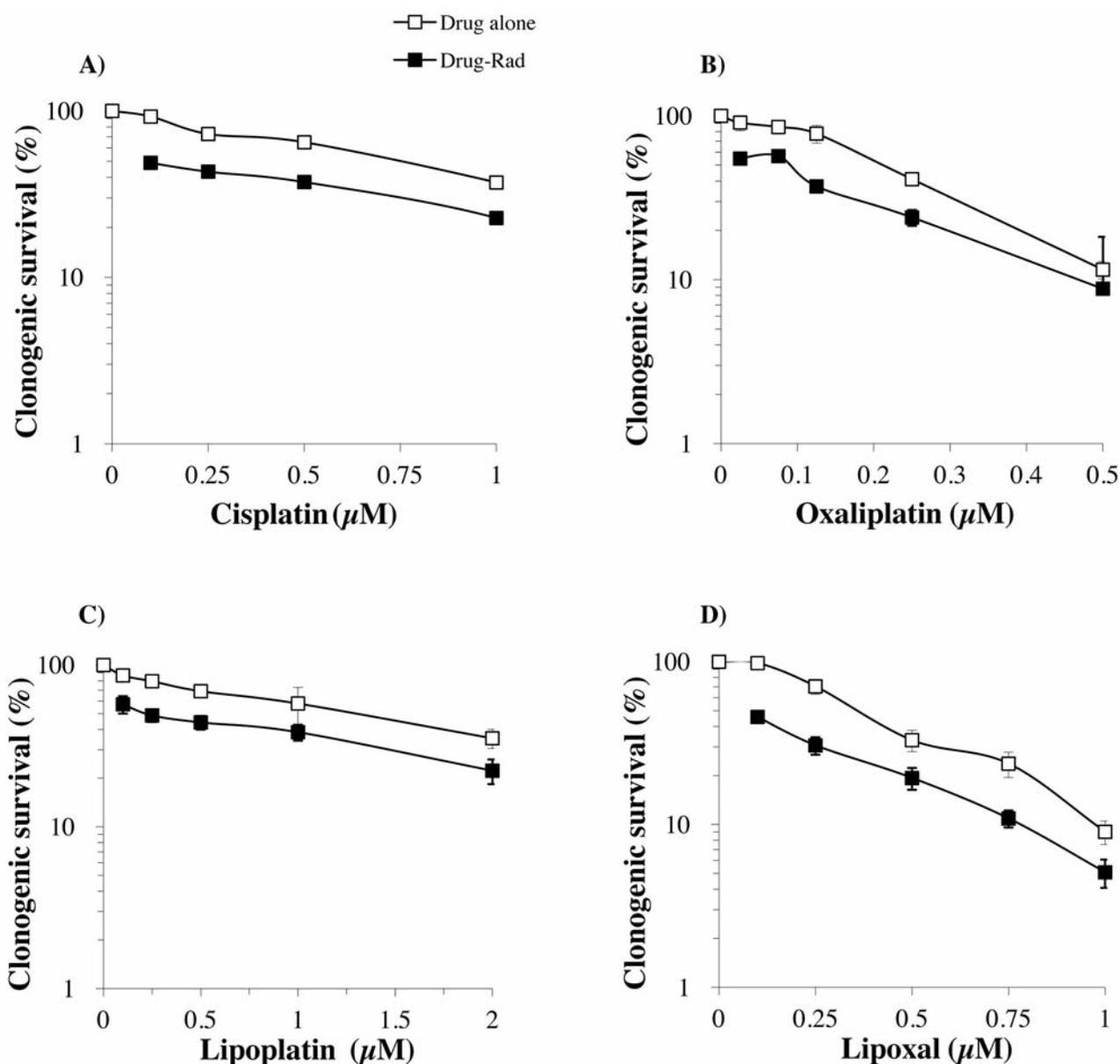


Figure 3. Response curves for chemoradio-therapy on HCT116 cells at 48 h incubation time. (□) Cells were exposed to various concentrations of each platinum derivative alone, or (■) in combination with gamma radiation (2.3 Gy). The concentration of the drugs is based on the IC_{50} values, which were previously obtained from the clonogenic assay at 48 h of incubation. Each point represents the mean of 3 independent experiments and the bars indicate the S.D.

and 0.77, respectively); but this gave only an additive effect with 48 h (CI=1.04, 1.09 and 1.01, respectively).

Cell cycle analysis. The cell-cycle progression of HCT116 cells exposed to four platinum drugs for 8 h and 48 h of incubation time with and without radiation treatment were observed as shown in Figure 6A and 6B. Radiation treatment increased the accumulation of the cells in the G_0/G_1 and G_2/M (1.15- and 1.22-times), while decreased the S phase

fraction (1.63-times) compared to the control group ($57.33\pm 3.26\%$, $29.77\pm 3.18\%$, $13.03\pm 1.02\%$ for G_0/G_1 , S and G_2/M phase, respectively).

Platinum chemotherapy: After 8 h incubation, the cell accumulation of the G_0/G_1 , S and G_2/M phase were observed for cisplatin, oxaliplatin, Lipoplatin™ and Lipoxal™, respectively, at $56.88\pm 1.58\%$, $50.57\pm 1.06\%$, $47.32\pm 3.00\%$ and $57.14\pm 5.86\%$, respectively; $22.45\pm 0.81\%$, $23.09\pm 1.98\%$, $35.10\pm 1.31\%$ and $28.32\pm 3.86\%$, respectively; and $20.67\pm 1.01\%$,

26.38±1.48%, 17.58±1.73% and 14.29±2.36%, respectively. These values of cell accumulation in the G₀/G₁ were increased at about 1.4-, 1.46-, 1.68- and 1.29-times the values, respectively, when the incubation time was increased to 48 h. For the cell accumulation in the S and G₂/M phase the were reduced about 2.30-, 1.48-, 3.88- and 1.47-times of magnitude, respectively; and 1.97-, 2.56-, 1.70- and 1.32-times, respectively, when the incubation time was increased to 48 h.

The chemoradiation combination: After combined radiation treatment with cisplatin, oxaliplatin, Lipoplatin™ and Lipoxal™ administration, the cell accumulation in the G₀/G₁ phase for 8 h was increased, while a slightly-reduced cell accumulation was observed after 48 h incubation. The reduction of cell accumulation in the S phase, after combined drug and radiation, did not change after the combination at 48 h of drug incubation. The similar trend of the increase of cells in the G₂/M phase, for 8 h and 48 h drug incubation, was observed.

Induction of apoptotic cells. To investigate whether there was induction of apoptosis in cells after the combined treatment, the cells in the subdiploid (sub-G₁/G₀) phase were analysed (Figure 7A and 7B).

Platinum-drug chemotherapy: After 8 h of incubation with cisplatin, oxaliplatin, Lipoplatin™ and Lipoxal™, the percentage of the apoptotic cells was observed to be 1.54%, 1.68%, 0.76% and 1.70%, respectively. These percentages of four tested drugs were reduced about at 10.3-, 8.0-, 1.0- and 1.2-times of magnitude, respectively, when the incubation time was increased to 48 h.

Chemoradiation combination: The percentages of apoptotic cells were considerably induced when HCT 116 cells were incubated with the tested drugs for 8 h prior to radiation treatment. The apoptotic cells were reduced about at 1.1-, 2.2-, 1.6- and 1.3-times of magnitude for combination of radiation and cisplatin, oxaliplatin, Lipoplatin™ and Lipoxal™, respectively, when the time of incubation was increased to 48 h.

Discussion

Despite their narrow therapeutic index and cumulative toxicity, platinum compounds are widely used as chemotherapeutic agents. Almost all current cisplatin-based chemo-radiotherapy protocols consist of daily administration of radiotherapy, with intermittent administration of the agent. This implies that tumor and healthy tissues are exposed to radiotherapy after different periods of administration to cisplatin, and that the drug level varies overtime. The same scheme is also found in most protocols associating oxaliplatin and 5-FU regimens in the chemoradiotherapy treatment of rectal cancer.

The cytotoxicity of platinum-based drugs is dose- and time-dependent (5, 22). An effective combination of radiation and chemotherapy is expected to lead to synergism or at least yield an additive effect on tumor cells. Bentzen and co-worker have

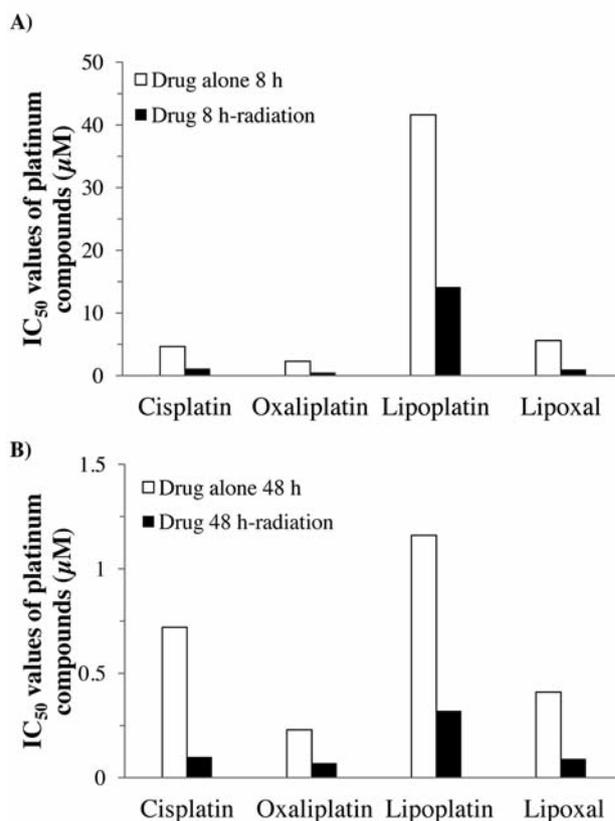


Figure 4. Combination of cisplatin, oxaliplatin and their liposomal formulation (Lipoplatin™ and Lipoxal™) with radiation decreases the IC₅₀ values of the platinum compounds incubated with HCT116 cells for A) 8 h with or without radiation and B) 48 h with or without radiation.

suggested that for maximal cytotoxic enhancement the drug must be present at the time of irradiation to modify the initial stage of radiation-induced cell killing or repair (23). Adequate information concerning the pharmacokinetics of each used drug, as well as the intra-tumor drug distribution could therefore help optimizing the treatment schedule.

We previously studied the kinetics of cisplatin and oxaliplatin uptake and that of their liposomal formulation uptake in the human colorectal cancer cell line HCT116 (12), in order to determine the amount of platinum in different tumor cell compartments and that of DNA-bound platinum at different time intervals after administration. For the free cisplatin and oxaliplatin, the rapid initial accumulation in DNA reached respectively 0.16 ng Pt/µg DNA and 0.32 ng Pt/µg DNA after 8 h of incubation. The levels of platinum DNA adducts were progressively reduced as a function of time, down to a minimum at 48 h. More than 90% of the drugs were accumulated in the cytoplasm after 48 h of incubation with the cells. Lipoplatin™ and Lipoxal™ resulted in a generally more than 10-fold lower distribution of platinum in DNA compared

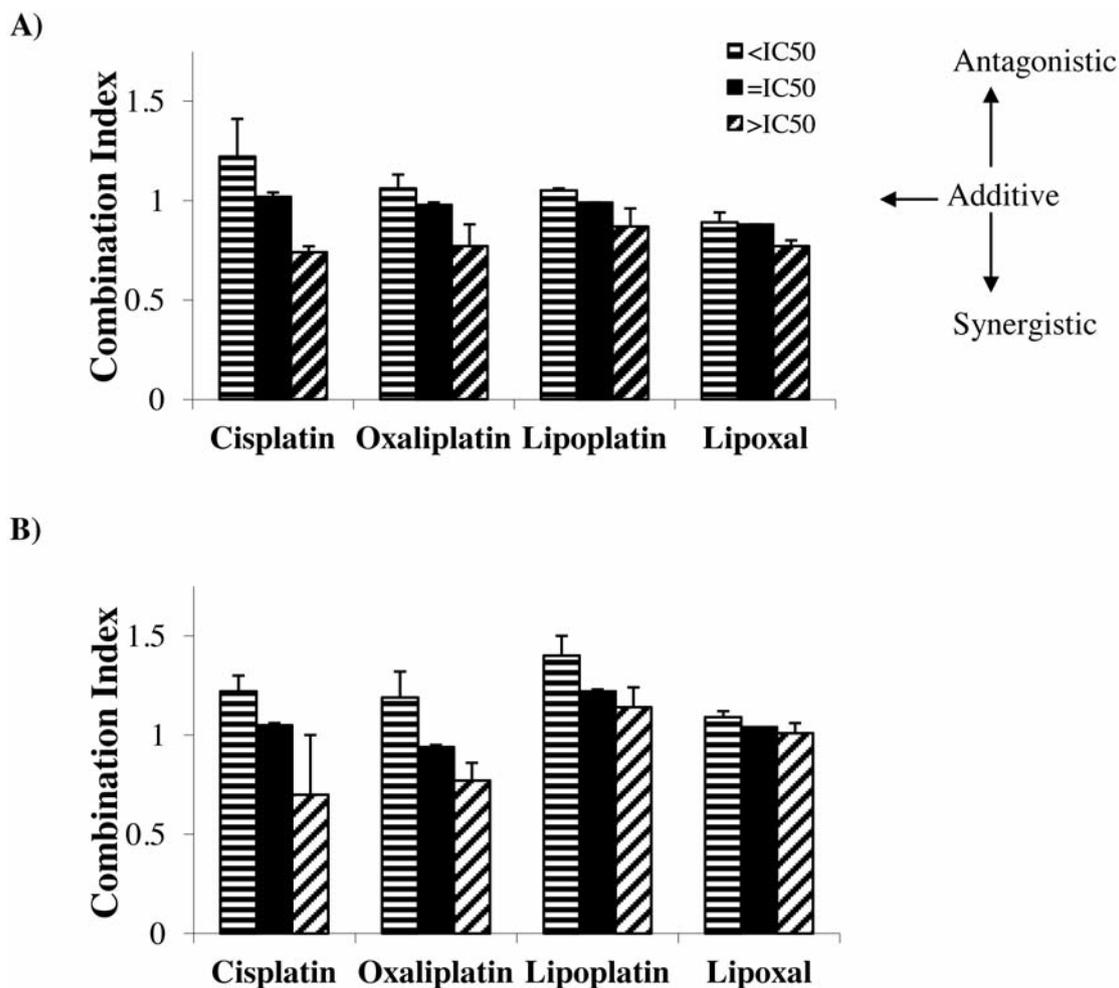


Figure 5. The combination index (CI) of chemo-radiotherapy on HCT116 cells at 8 and 48 h of incubation time. Column (▨), (■) and (▩) indicate CI values at the concentration of platinum drugs lower, higher and equal to the IC_{50} values.

to their free formulation. The results of cellular uptake and cytoplasmic/DNA distribution of cisplatin and oxaliplatin and their liposomal formulation in HCT116 suggested the point of maximum DNA-platinum adducts formation to be at 8 h.

In addition, the radiosensitivity is a function of the cell-cycle. The radiosensitivity is pronounced when the cells are in the G_0/G_1 and G_2/M phase, while cells are radioresistant when they are in the S phase. The G_1 arrest showed that the majority of the cells after drug treatment for both 8 h and 48 h incubation with and without radiation, were accumulated in the G_1 phase. Our results are similar to those of previous studies (24-26). Moreover, the cell accumulation in the G_2/M phase which is the most radiosensitive phase, was mostly observed after 8 h of drug incubation with and without radiation, while no significant change was observed after 48 h incubation. These results accompanied by a higher proportion of apoptotic cells after 8 h of incubation than after drug exposure for 48 h

of incubation. Thus, we chose to test the effect of associating radiotherapy and chemotherapy at 8 h and 48 h to clarify if the maximum DNA-Pt could improve synergism.

Clonogenic assays after 8 h of incubation indicated that the concentration of cisplatin and oxaliplatin, needed to obtain the same level of cytotoxicity, was approximately 3-10 times lower than their liposomal formulation. When the incubation period is increased to 48 h, there were still about a factor of two differences in the IC_{50} values between cisplatin and oxaliplatin, compared to their liposomal formulations. This confirms our initial results on the pharmacokinetics of platinum drugs (12) where the cells were less sensitive to the liposomal-platinum formulation than to the free-platinum formulation. For all tested drugs, whether after 8 h or 48 h of incubation, the association of radiotherapy reduced the IC_{50} value and increased the efficiency of HCT116 cell killing by a factor of 3 to 7.

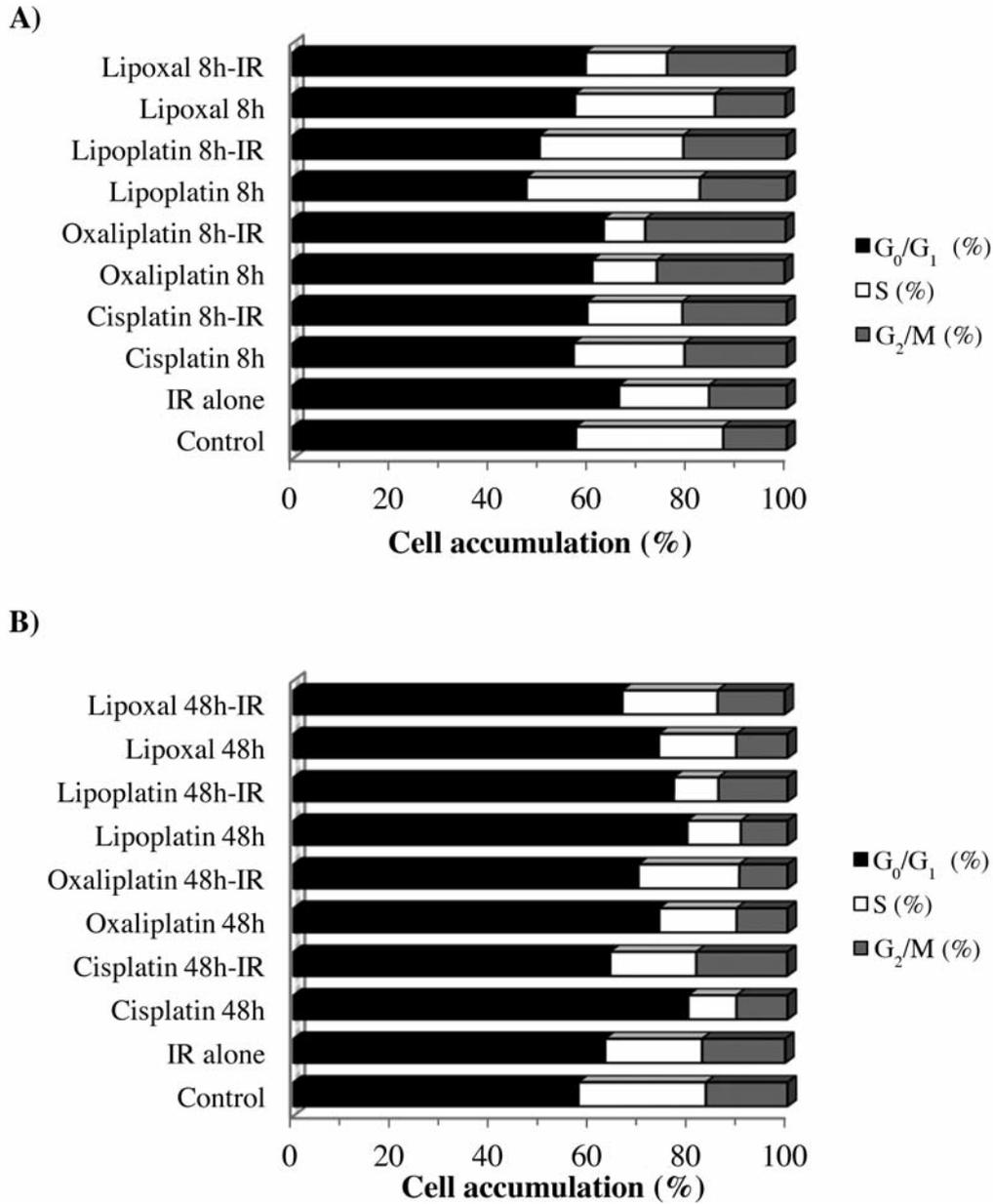


Figure 6. Flow cytometric measurement of cell-cycle distribution of the HCT116 cells after cisplatin, oxaliplatin, Lipoplatin™ and Lipoxal™ administration for A) 8 h and B) 48 h incubation, followed by irradiation at 2.3 Gy. The concentration of the drugs is based on the IC₅₀ values, which were previously obtained from the clonogenic assay at 8 h or 48 h of incubation. Mean of three experiments is shown.

As each of these drugs remain for a long time in the target tissues, while the patient is given radiotherapy daily, the differences of drug concentrations and the time intervals between drug administration and radiation treatment could cause a difference in the combined effect. Our results confirm our hypothesis that radiotherapy at various times after injection of the platinum drugs, at different

concentrations, could lead to different outcomes. For cisplatin, a drug concentration higher than IC₅₀ is synergic, while a lower than IC₅₀ concentration is antagonistic. Although, cisplatin showed a great improvement in synergism at high concentrations, its severe toxicity is of considerable concern. For oxaliplatin, exposure to a concentration above IC₅₀ for 8 h is synergic, while the

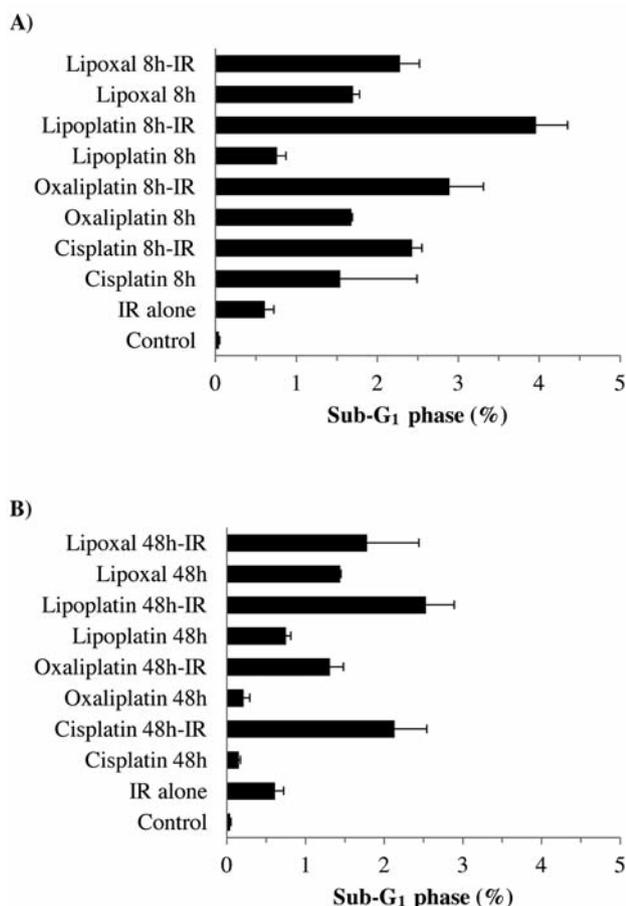


Figure 7. Induction of apoptosis by cisplatin, oxaliplatin, Lipoplatin™ and Lipoxal™, in combination with radiation. The number of apoptotic cells was quantified by PI staining and flow cytometric analysis in HCT116 cells treated with A) 8 h and B) 48 h of incubation with platinum drugs-alone or in combination with radiation at 2.3 Gy. The concentration of the drugs is based on the IC_{50} values, which were previously obtained from the clonogenic assay at 8 h or 48 h of incubation. Mean of 3 independent experiments is shown and the bars indicate the S.D.

exposure to oxaliplatin (at any concentration) for 48 h is antagonistic. Lipoplatin™ showed a similar trend as the ones observed with cisplatin, but Lipoxal™ differed significantly from its parent drugs. These results also suggested that time-dependence on irradiation are more important to reach the synergism in the case of oxaliplatin and Lipoxal™ than for cisplatin and Lipoplatin™.

Several potential mechanisms of platinum-mediated radiation sensitization have been reported (5, 10, 27) to rationalize the synergic and additive effects of chemoradiation treatment. Zheng and co-worker clearly demonstrated that there is a significant increase of single-

and double-strand breaks of DNA, when DNA is irradiated in the presence of cisplatin (10). In addition, it has been proposed that ionizing radiation caused higher uptake rates, probably due to an increased permeability of the cells membrane (28, 29). Some authors suggested that a sufficient number of platinum atoms must accumulate in the cancer cell to reach a radiosensitizing effect. Gabriel and co-workers showed the relationship between cell uptake and radiosensitizing potential (30). Liposomal formulation showed promising results in order to increase the level of cell uptake, compared to their free platinum form. High accumulation of liposomes in the cancer cells could be of considerable value to increase the benefit of chemotherapy when it is combined with radiotherapy.

An antagonistic effect was proposed to be associated with the efficiency of DNA repair, as well as, alterations in cell oxygenation and the cellular levels of thiol groups (31, 32). The prolonged time of drug incubation could lead to the reduction of platinum accumulation and Pt-DNA adducts. An antagonistic effect was clearly observed when HCT116 cells were treated with oxaliplatin and Lipoxal™ at a time interval of 48 h before irradiation. Importantly in the clinical treatment, a typical radiation treatment schedule is 2 Gy irradiations each day for 5 days a week (33), irradiation at the time point that displayed low level of platinum concentrations in the cell would lead to an antagonistic effect.

Conclusion

The present study provides information about the schedule dependence of the synergism of platinum-based drugs and radiation. Confirmation of these data and their validation in animal models can have a tremendous impact on designing future platinum-based chemo-radiotherapy protocols. Associating radiotherapy to the time intervals of maximum synergism could improve efficacy and limit toxicity of chemotherapeutic agents.

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