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Cancer Chemotherapy and Pharmacology

Meeting Report

C.R.O.S. Conference on Combined Modalities Chemotherapy/Radiotherapy

Hilton Head Island, South Carolina, November 15–18, 1978

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The Committee for Radiation Oncology Studies at the NCI supported a conference on Combined Modalities: Chemotherapy/Radiotherapy, which was held on Hilton Head Island, South Carolina, 15–18 November 1978. The chairman of the organizing committee was Dr. Theodore Phillips of the University of California, San Francisco.

The conference was approached through emphasis on five drugs and their interaction with irradiation. The five drugs were adriamycin, *cis*-platinum diamine dichloride, cyclophosphamide, nitrosoureas, and bleomycin. Abstracts were submitted on these drugs and were broken down into six categories:

1) Biochemistry,
2) In vitro experimental data,
3) In vivo experimental data,
4) Normal tissue effects,
5) Pharmacology,
6) Clinical.

Sessions were held on each drug, moving through the accepted abstracts in the above order, with discussion, and ending with a drug summary by one of the two invited session co-chairmen.

The conference began with a plenary session in which invited speakers addressed topics such as experimental design considerations for clinical and experimental studies, terminology, host response, standardization of end points for in vitro and in vivo systems, and normal tissue systems, cell kinetics, pharmacology, and the timing of assays. At the end of the conference summary presentations were made, again by invited speakers.

One of the major problems throughout the field of oncology, including combined treatment with drugs and irradiation, is the correlation of experimental and clinical data. What tends to happen is that the experimentalist and the clinician speak different languages (Table 1) and work independently of each other, paying lip service only to the importance of their communication. What is needed are data from experimental systems that can help the clinician design more effective regimens. This can only occur if the clinician communicates with the experimentalist about the strategies and problems he is involved with. There should be dynamic interaction and questions so that new stimulus and ideas are moving bilaterally.

Table 1. Some differences between experimental and clinical combined-modality data as regards chemotherapy plus radiotherapy

	Experimental	Clinical
1.	Designed for prospective test of one aspect of the interaction, i.e., antitumor effect or host tolerance in a single tissue	Designed to improve therapeutic index in treatment. Individual interactions are retrospectively analyzed
2.	All important factors controlled	Great heterogeneity of important factors
3.	Usually interaction of single drug with single dose X-ray	Many times multiple drugs with fractionated X-ray
4.	End points of in vivo systems: growth, delay tumor regression, survival downgraded	Clinical end points: regression, disease-free survival, and overall survival; survival highly important
5.	System orientation	Disease orientation
6.	Rational design	Empirical design

One of the major problems in communication regards terminology. The clinician approaches the problem pragmatically, recognizing that cure requires local control and/or metastatic control. Since irradiation, as commonly used, is a local modality, by itself it can only achieve local control. The addition of drugs to radiotherapy can have two strategic potentials. One potential is to eradicate tumor cells outside of the irradiated field. This is defined as adjuvant use of the drugs. It does not assume any biological interaction occurring between the drugs and X-ray, as regards tumor cell kill. There may

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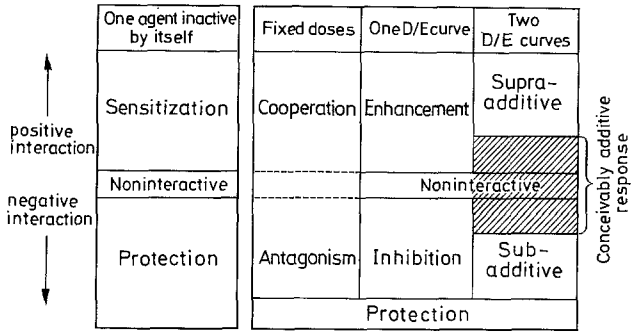

Fig. 1. Terms suggested for description of effects achieved with more than one agent

well be toxicologic interaction, however. The second potential has drugs enhancing the local control of radiotherapy. The clinician calls this sensitization, but in a truly biological sense this may not be occurring. The drugs may be killing cells untouched by the X-ray or may only be giving additive damage. If permanent local control is achieved clinically with drugs and X-ray, while X-ray alone cannot accomplish this, this is clinical potentiation of effect. It may not be possible, in a biological sense, to dissect out exactly the interactions that occurred at the tumor cell level.

The experimentalist, however, likes to be very careful about his terminology, and since agreement is not uniform this engenders vigorous and occasionally heated and prolonged debate. How do we define effects that may be $2 + 2 = 3$, $2 + 2 = 4$ or $2 + 2 = 5$? At the meeting, G. Gordon Steel postulated a veriety of terms, which are illustrated in Figure 1 and defined in a glossary (Table 2).

Perhaps the most important drug discussed at the meeting was adriamycin. This is because adriamycin has such a broad spectrum of activity and such a wide potential for interaction with radiation. There are at least ten tumors in which adriamycin is being used clinically in combination with X-ray (Table 3). As can be seen, in none is adriamycin being used as a single agent. Therefore, all the complexities of combining one drug with X-ray are magnified several fold by the use of those combination regimens. This greatly complicates the possibility of developing meaningful clinical correlations with the experimental data base. At the meeting most of the experimental data related to interactions of the single agent anthracycline with a single dose of X-ray. These systems mostly looked at the interaction on a single aspect of the complex that must take place when patients are treated.

Ross, Glaubiger, Zwelling, and Kohn discussed the relationship between cytoxicity and DNA strand breakage produced by adriamycin and other intercalating agents. They conclude at this time that intercalating agents cause the formation of protein associated DNA breaks, but the role of these breaks in cytotoxicity is unclear.

Table 2. Glossary

Antagonism	Apparent negative interaction between agents in a situation where dose-response curves are not available
Cooperation	Apparent positive interaction between agents in a situation where dose-response curves are not available
Enhancement	The situation in which the dose-response curve to one agent appears to be made steeper by combination with another agent
Inhibition	The situation in which the dose-response curve to one agent appears to be made less steep by combination with another agent
Interaction	Any situation in which there is evidence for one agent influencing response to another
Noninteractive	The situation in which agents appear to act independently. In simple terms this is what has often been called 'additive'
Protection	The situation in which a combination produces less effect than one agent alone. This is clearest when an inactive agent reduces the effect of an active agent, but can occur with two active agents
Sensitisation	The increase the response to a cytotoxic agent by the administration of an agent that is itself inactive
Spatial cooperation	The improvement in therapeutic result achieved by using chemotherapy and radiotherapy to treat disease in different anatomic sites
Sub-additive/ Supra-additive	Terms to be used when full dose-response curves are available for the separate agents. When the combined response is consistent with some interpretation of the dose-response curves we may regard this as conceivably additive. Sub- and supra-additive response are then indications of strong interaction

Sagone, from Ohio State, raised the possibility that the cardiac toxicity of adriamycin may be related to the intracellular generation of reactive oxygen metabolites by the drug. This group presented studies, in abstract form, indicating that adriamycin injury to human lymphocytes may not be dependent on the generation of these reactive oxygen metabolites. This raised the possibility to them that antioxidants might diminish cardiac toxicity while not hindering cell kill of lymphoid malignant tissue.

Kimler and Leeper are cautiously optimistic about in vitro studies helping to predict the optimal sequence of adriamycin and X-ray. They studied the interaction in Chinese hamster ovaray cells and saw a positive interaction in terms of a diminished surviving fraction.

Belli and Harris have studied adriamycin resistance and how this affects radiation response in V79 mammal-

Table 3. Ten tumors in which adriamycin is currently being used clinically with X-ray therapy

Tumor	Other drugs being used in combination with adriamycin
1. Soft tissue and bone sarcomas	Actionomycin, Cyclophosphamide, Vincristine, Dacarbazine
2. Lung cancer	Cyclophosphamide, Vincristine, CCNU, methotrexate, VP-16, *cis*-platinum
3. Hodgkin's disease	Bleomycin, Vinblastine, Dacarbazine, Nitrogen mustard, Vincristine, Procarbazine, Prednisone
4. Non-Hodgkin's lymphoma	Cyclophosphamide, Vincristine, Prednisone, Bleomycin
5. Ovarian cancer	Cyclophosphamide
6. Breast cancer	Cyclophosphamide, 5-fluorouracil
7. Wilm's tumor	Actinomycin D, Vincristine
8. Embryonal rhabdomyosarcoma	Actinomycin D, Cyclophosphamide, Vincristine
9. Ewing's sarcoma	Cyclophosphamide, Vincristine
10. Hepatic metastases, gastrointestinal cancer	Adriamycin, Methotrexate

ian cells. Their data imply that the phenotypic expression of adriamycin resistance may be accompanied by a deficiency in X-ray damage repair. This is important, since adriamycin does not substantially inhibit the repair of sublethal or potentially lethal radiation injury in V79 cells. The same group also presented data showing that uptake and release of drug into V79 cells is a critical factor required for understanding adriamycin–X-ray interactions. Adriamycin-resistant V79 cells had markedly decreased levels of drug uptake into the cytoplasm and DNA compared with parent cell lines. Plateau phase cells were significantly more resistant to adriamycin killing than exponential phase cells and had significantly less drug uptake.

Ransom, from St. Jude Children's Research Hospital, reported on delayed gastrointestinal complications following combined drug and irradiation in 15 patients with retroperitoneal rhabdomyosarcomas. The chemotherapy included vincristine-actinomycin D alternating weekly with cyclophosphamide-adriamycin. This was followed by CO^{co} X-ray therapy to documented areas of tumor extension, including the pelvis, para-aortic nodes, or abdomen. Combination chemotherapy was continued during the radiotherapy interval and for 18 months in the absence of progressive disease or life-threatening toxicity. In five of the fifteen patients major gastrointestinal toxicity was observed, with three deaths occurring after the acute onset of small bowel obstruction. The histopathology in each case was consistent with severe radiation enteritis or proctitis. Since past regimens using actinomycin D, cyclophosphamide, and vincristine did not show this, the assumption is that adriamycin is responsible. This may be adriamycin alone or in combination with the other drugs, especially actinomycin D.

Schenken et al., from Allegheny General Hospital, reported at the meeting that increased gastrointestinal radiosensitivity was observed 3–7 weeks after adriamycin treatment. When adriamycin was given to male DBA/2 mice, the ability of jejunal mucosa to respond proliferatively to a 1000-R test dose of abdominal radiation was progressively diminished from day 14 on. Proliferative peaks were reduced, as was integrated cell production. While the crypt survival did not change, the acute $LD_{50/7}$ was reduced to half, and tolerance to various fractionated exposures was reduced by as much as 75%. The data of Schenken et al. suggest that adriamycin caused a delayed proliferative impediment, probably due to either stem cell damage or progressive compromise of secondary support systems such as jejunal vascularity.

Ross and Phillips presented data on the intestinal crypt cell assay in LAF mice after adriamycin and radiation. Their results revealed that adriamycin is toxic to intestinal crypt cells, but does not inhibit the repair of radiation injury.

Kumar et al., from St. Jude, have studied the intestinal radiosensitization interaction of adriamycin and actinomycin D in BDF mice. Their data show that adriamycin appears to potentiate the radiosensitizing effect of actinomycin D on intestinal epithelium.

In terms of studying the antitumor effect of combined adriamycin and irradiation experimentally, the in vivo system used by most of the investigators reporting at the meeting is the EMT-6 tumor. This appears to be a versatile system that can be used in a variety of ways, one of which allows a test of cell kill by in vitro techniques. The data are complicated by the fact that the system differs in different institutions. The EMT-6 line used at Stanford is significantly immunogenic, while the one used at the University of California, San Francisco (UCSF), is not. Unfortunately, this system is not very responsive to adriamycin. In fact, J. C. Harris and Shrieve, at UCSF, reported that EMT-6 cells growing in mice are not only quite resistant to the drug, but in addition do not show radiosensitization with the drug. Interestingly, EMT-6 cells in vitro are sensitive to adriamycin. The drug is equally effective against both euoxic and acutely hypoxic cells in a 1-h test at 37° C. Despite this, radiosensitization cannot be demonstrated in the cell cultures.

Fu, Begs, and Phillips reported on the interaction of adriamycin and radiation in vivo in three different sizes of the EMT-6 tumor, i.e., 1-cm flank tumors, 2-mm macroscopic lung nodules, and microscopic pulmonary me-

tastases. For the flank tumors the drug was slightly more effective with IV than with IP administration when survival was assayed in vitro 24 h after drug therapy. When adriamycin was combined with radiation, there was no significant difference in suvival level with variation of the time interval between drug and radiation administration from −48 h to +48 h. When flank tumors were treated with drug and followed after 24 h by graded doses of X-ray, an additive killing effect was seen at low radiation doses, but at high doses radiation alone was equally effective. In the microscopic pulmonary metastases the effect of drug and X-ray was strictly additive.

Twentyman and Kallman studied adriamycin (6 mg/kg) and 1200 rads in EMT-6, KHT, and RIF-1 mass tumor systems. The study was designed to determine the effect of different time intervals between the administration of drug and X-ray. The tumors all grew 1 m in the gastrocnemius muscle and the end point is the time at which the volume reaches two to four times its initial value. Adriamycin did not produce a significant delay in the growth of any of the tumors. The growth delays produced by the drug-X-ray combinations were not significantly longer than those produced by radiation alone.

Sutherland, from Rochester, set out data on the uptake and response of EMT-6 tumor cells to adriamycin when the cells were grown as single cells, multicell spheroids, or solid tumors. The cells in the spheroids were significantly more resistant than the cells in either exponential or plateau phase growth in monolayers. The data indicate that the resistance of cells in spheroids may be related to several phenomena: (1) poor drug diffusion into the spheroid, (2) differences in drug uptake between cycling and noncycling cell populations, (3) intercellular contact and/or heterogeneous inter- or intracellular drug distribution. The EMT-6 cells grown as a solid tumor were also resistant to adriamycin. Sutherland pointed out the necessity of evaluating carefully the different factors that modify the response of tumors to drugs in order to understand the mechanisms of response after combined-modality treatments. Unfortunately it is hardly possible to elucidate these in clinical studies, as was pointed out earlier. What is clear, however, to paraphrase Gertrude Stein, is that the EMT-6 is not the EMT-6 is not the EMT-6.

In summary, the use of adriamycin in in vitro studies has yielded data that indicate the importance of cell-cycle characteristics, tumor-cell density, and induced resistance as factors that can impact upon the interaction of adriamycin and X-ray. The ability to translate this directly to clinical application is hampered drastically by our lack of knowledge about human internal solid tumor kinetics, cell density, and the ratio of hypoxic to euoxic cells. In addition, the classic techniques of pharmacokinetics measure blood levels but tell us nothing about the concentration in time of the active moieties at the tumor cell level. Without the kinetic and pharmacologic data the clinician is in an empiric black box, and at this time can only use experimental data to soothe his anxiety about his empiricism.

The in vivo studies are hampered by the failure of the EMT-6 tumor to be meaningfully responsive to adriamycin. A system to study a drug and X-ray interaction adequately should ideally be responsive to both the drug and irradiation. The EMT-6 appears to be analogous for radiobiologists to the L1210 for experimental chemotherapists. The systems are well known and easy to use and manipulate. While the L1210 may be adequate for screening new structures, it is not nearly as effective for analogues, combination chemotherapy, and schedule dependency studies. The EMT-6 system appears adequate to elucidate certain radiobiologic principles and parameters. It may be that the system is being stressed when combined modality is imposed upon it with a drug to which it is unresponsive.

Toxicologically, adriamycin and X-ray appear to interact positively as regards cardiac toxicity and gastrointestinal toxicity. In these situations the experimental normal tissue systems appear to be helpful. The data of Schenken predicted the unfortunate experience at the St. Jude. The experimental studies at St. Jude indicate that the problem may be caused by the combination of adriamycin with actinomycin D in the face of gut radiation. This can now be tested clinically. The era of combined-modality treatment has taken the medical oncologists further into the arena of chronic organ site toxicity, where the radiation oncologist has long been expert. Model systems for normal tissue damage should be given high priority in future studies.

Similar papers were presented on other drugs, such as *cis*-platinum, cyclophosphamide, bleomycin, nitrosoureas, and miscellaneous agents. Space does not permit a rundown of these. Since the proceedings will be published in the International Journal of Radiation Oncology, these will be available to all. The meeting was an extremely important one in that it continued the four-way dialogues between the radiation oncologists, radiobiologists, medical oncologists, and experimental chemotherapists. If combined-modality treatment is to achieve its full potential, then all four will have to work together as a concerted team.

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