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COMMENTARY

INTERACTIONS OF IONISING RADIATION AND CANCER CHEMOTHERAPY AGENTS

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This Commentary presents a Clinical Scientist's view of the state of the art of drug–radiation interaction with the intention of stimulating further research into both the fundamental mechanisms and the applied biology of the subject.

In a modern oncology centre cancer patients will receive more than one of the therapeutic modalities of surgery, radiation therapy and chemotherapy (plus the possibility of immunotherapy when this is better understood). There is increasing recognition of the fact that the total mass of malignant cells [1] can only be reduced below that level required for cure, or prolonged remission, if combination therapy is employed. Surgical excision of an accessible tumour will obviously reduce the cell mass to a minimum but residual disease is frequently present and this requires ablation. Microscopical deposits of malignant cells may be present both at the site of the primary tumour and elsewhere in the body. Chemotherapy or total body radiation will be required for the eradication of generalised disease. A combination of ionising radiation and chemotherapy will be most useful in the treatment of those larger deposits of tumour cells which remain near the primary site. The present search is for a combination therapy which will be more cytotoxic to malignant disease but no more damaging to normal tissues.

In this commentary on the problem, the word "interaction" will be given no more specific meaning than that the action of radiation and drug on the biological system are not completely independent. Terms like synergism, potentiation, dose-modifying factor or additive effect have all been used in the literature. Because the precise mechanism of a combined action is not always known and the two methods of treatment can not always be shown to enhance the cytotoxic effect in a systematic manner, the term synergism is rarely appropriate. Potentiation is a more acceptable term to describe the phenomenon when a combination of doses of two different cytotoxic agents produces an effect which is more than would be expected if those doses of the two agents were used separately.

This obvious definition needs to be restated because so much of the literature on combination therapy contains reports which show no more than a simple additive effect; or sometimes even an effect on the biological system which is less than would be expected from addition of the two cytotoxic regimes. This possibility is illustrated in Fig. 1, where combination of radiation and the platinum coordination complex was shown to be less than additive when low doses were used but potentiation was demonstrated at the highest doses.

The results demonstrate that combined therapy enhanced the therapeutic effect when C-Pt was administered 4 hr prior to γ-radiation to mice inoculated intraperitoneally with P388 cells over a wide range of combinations. The maximal increase in life spans was 64 per cent for 4 mg/kg C-Pt alone and 36 per cent for 600 rads of γ-radiation alone. Combined therapy produced a 114 per cent increase in life span (% ILS). Quite obviously, the search is for regimes which produce *more* than an additive cytotoxic effect on malignant tissue, although a lesser effect upon normal tissues would equally obviously be desirable. (In the rest of this commentary the word "radiation" will be used to mean any form of ionising radiation—usually of low LET—and the word "drug" will mean one of the recognised cancer chemotherapy agents.)

MECHANISMS

The combination of radiation and drug may be more effective because of one or more of the following

Combined therapy studies vs P388*

cis-Pt diamine dichloride**
Mg/kg per injection

γ-Radiation, rads	0	0.5	1	2	4
	% ILS				
0	0	18	27	36	64
100	9	18	27	46	73
200	18	27	46	55	73
400	36	55	64	68	91
600	36	64	46	82	114

*P388 10^6 cells i.p.
**Administered 4 hr prior γ-radiation

Fig. 1. Combined therapy of P388 leukaemia in CDF_1 mice using *cis*-platinum diamine dichloride (C-Pt) and ^{60}Co γ-irradiation (data of Wodinsky *et al.* [2])

mechanisms: (a) interaction may occur due to presence of the drug during irradiation (b) the drug may interfere with repair of radiation damage (c) there may be some differential effect upon the proliferation kinetics of tumour and/or normal cells—this may already be a differential property of the cells [3]–or it may result from the response of the cell populations to drug and/or radiation. Evidence of the operation of these and other possible various mechanisms will add more credence to reports of enhanced cell killing by a drug–radiation combination. Equally importantly, they will indicate lines of research which might be more successful than the many random combinations which have just been "given a trial" without any particular scientific hypothesis. Time may then be saved and cancer patients may be spared unnecessary toxicity.

The presence of the drug during radiation should ideally be demonstrated by standard biochemical and pharmacological techniques [4–8].

If the active moiety of the drug molecule can be labelled with radioactive isotope, this demonstration is relatively simple (although invasive methods of sampling such as biopsies are not always possible or ethically desirable with human subjects). If the active moiety cannot be labelled or tissue samples cannot be studied then indirect evidence of drug binding will have to suffice, but doubt must then remain if or how the drug interacted with the radiation. It will also be important to know whether the drug binds to the target molecules in the cell following passive diffusion or some mechanism of active transport [9–11].

Before studying repair of radiation damage in cells exposed to drug–radiation combination it is necessary to know the pattern of repair in that cell following damage by radiation alone and drug alone. Following radiation, it is known that the timing of events within a cell which is attempting to recover from damage to its DNA will depend upon four factors: (1) the nature and number of lesions produced in its DNA (2) the ability of the cell to perform those different types of repair required by such lesions (3) the growth conditions at the time which may help or hinder the cell's different repair mechanisms (4) the efficiency of each repair event in restoring the full competence of the original DNA in both its transcriptional and replicative roles.

The same sort of information should be sought for each drug before the repair mechanism can be expected to be understood in drug radiation combinations. At the present time there is a relative dearth of information, but such data as are available show that many drugs may reduce repair capacity following radiation. It is not clear if and how the molecular biological evidence for the repair of DNA strand breakage can be correlated with the biological phenomenon usually termed "recovery from sublethal damage" [12–14] but it is known that the drug

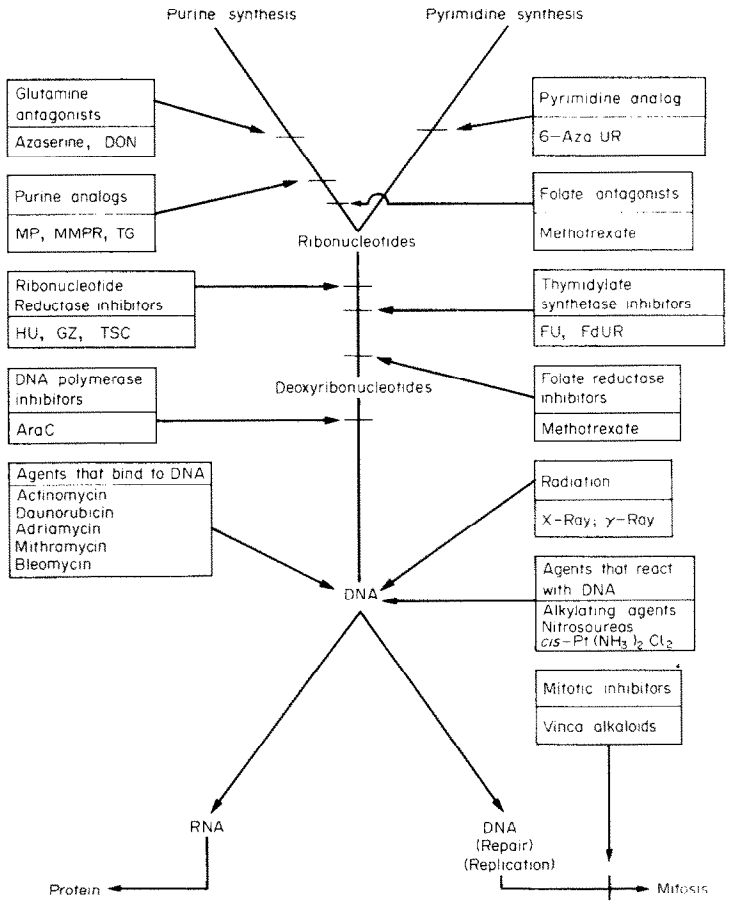

Fig. 2. Loci of action of anticancer agents (from Brockman [18])

actinomycin D which inhibits RNA synthesis also inhibits "Elkind recovery" [15, 16]. Other drugs which are known to reduce the amount of "Elkind recovery" include 5-fluorouracil, BUdR and IUdR [17], vinblastine and the platinum coordination complex (which will be discussed later as an example of methodology in drug–radiation studies).

Turning to the third mechanism, this is related to the proliferation kinetics of the cell populations affected by the drug–radiation combination treatment. This is the point in this review to state what has so far only been inferred—namely, that DNA is considered to be the prime target of both radiation and drug therapy. This may be a misleading generalisation as far as some cancer chemotherapeutic agents are concerned—some will undoubtedly bind to and damage organelles and molecules in the cell other than the nucleus and its DNA. The main objective of cytotoxic action in the context of cancer therapy must be loss of the proliferative capacity of malignant cells, however, and this parameter will be considered in this review to the exclusion of all other types of cellular malfunction which follow drug action. (It can already be stated that radiation in the therapeutic dose range damages DNA and its function more than any other cellular constituent.)

Figure 2 shows a schematic diagram of the synthetic pathway of cellular macromolecules with sites along it where many drugs are believed to act [18]. This diagram serves not only to list the commoner drugs but also to suggest a rationale for drug–radiation combinations. If radiation does indeed act primarily at the point indicated on the diagram then it would be reasonable to combine this with drugs which act at other points in the pathway. This hypothesis has sometimes been applied in the selection of drugs for multiple drug "cocktails". In the present example the selection of that particular site of action for radiation is suspect for two reasons; one general and one particular. The general reason is that radiation is delivered to all parts of a cell as randomly distributed ionising events. It can only be postulated that a particular site is relatively more sensitive to the same amount of radiation damage which other sites also receive. On the other hand, a specific biochemical target can be postulated for each drug and the schematic diagram in Fig. 2 may have more validity, although a particular objection is the placing of the platinum coordination complex and radiation at the same point, since combination studies (described below) show some potentiation. This may only reinforce the general point about the random distribution of radiation damage, however.

There is no reason to question the principle that multiple drug regimes should be designed on the basis that drugs used in combination should act by different mechanisms and that each drug should be given at a time during the cell cycle when that drug will exert its maximum effect (a point to be discussed in a later section, concerning proliferation kinetics). It has to be remembered, however, that although cell killing may perhaps occur only following a lethal event during one phase of the cell cycle (e.g. DNA synthesis), a drug which is taken into a cell and bound there irreversibly may ultimately cause death even though exposure of the cell to the drug occurred in some other phase of the cycle. For that reason many common agents should really be placed in the class of drugs which are not cell cycle-specific [19, 20]. On the other hand there need be no such misunderstanding about the effect of ionising radiation since this is a cytotoxic agent which is not "bound" irreversibly to cells.

TECHNIQUES

Demonstration that a particular drug–radiation combination has more than an additive effect requires parameters that can be applied to the cell population(s) of interest; at the cellular level *in vitro*, *in vivo* or in clinical practice. Without such quantitation, drug–radiation interaction cannot be proved and it is sad that so many clinical reports can be criticised in this respect. A realistic approach to this problem recognises that while *in vitro* cellular studies permit a high degree of quantitation and an optimal design of experiment, they may have minimal relevance to the clinical problem for which they are supposed to provide "model" systems. Animal model systems introduce not only the advantages of more "relevant" homeostatic and other environmental influences over the cell population(s), but also some disadvantage with respect to statistical control because of relatively much smaller numbers and often a biological variation that the use of inbred animals does not entirely remove. Dose–response studies are still possible, however, because there are no ethical problems.

Using *in vivo* techniques, the experiment depicted in Fig. 1 illustrates a suitable design with the dose–response assay both with each agent alone and for the combinations. Such an experiment will obviously have been preceded by detailed studies of the effect of each agent alone before this final design. The classical scientific approach of a hypothesis put to the test is sometimes forgotten. Time should be spent designing the experiment based upon earlier fundamental observations. Knowledge of the response of the whole animal and its relevant cell populations to both radiation (for which there are adequate data in the literature) and the drug (for which data may be very scanty indeed) is an essential prerequisite for the interpretation of the sort of experimental result shown in Fig. 1.

Cell cultures *in vitro* [21] permit the fundamental mechanism of drug–radiation interaction to be examined at the cellular level without interference from those homeostatic and other environmental factors which are so important *in vivo*, but so confusing to an understanding of the basic mechanism. Each environmental factor can be added to the system in turn, however, and there is no theoretical reason why the influence of all such homeostatic factors should not be studied—always assuming they are understood in themselves! Immunological mechanisms are now beginning to be understood [22] to the extent that humoral and cell-mediated immunity can be added to the culture system with some degree of quantitation. (The ephemeral chalones have yet to be quantitated.) An example of a study of drug–radiation interaction will be given later in which the monolayer cell culture system was used. Where drug diffusion studies require a more complex model system, more

akin to the *in vivo* situation of cell to cell contact, then the spheroidal cell culture technique is more appropriate [23].

CANCER CHEMOTHERAPY AGENTS

The list of drugs which have been used for cancer treatment grows daily and with it the problem of selecting those drugs which may be expected to show a more than additive effect when combined with radiation. A typical list was published by Carter and Soper [24] who discussed combinations of drugs and noted that successful regimes are based on the three criteria:

(1) Each drug in the combination should be active when used alone against the tumour.

(2) The drugs should have different mechanisms of action.

(3) The toxic effects of the drugs should not overlap, so that each can be given at or near its maximum tolerated dose.

These criteria can be directly applied to drug–radiation combinations. The second and third criteria have already been discussed with reference to Fig. 2. The first criterion is becoming increasingly difficult to establish in clinical practice because combination chemotherapy has proved so much more successful than single agent therapy that it may soon be regarded as almost unethical to use a single drug to evaluate tumour response. Animal and cell culture assays will continue to provide the basic data but introduction of a new drug will increasingly involve substitution of one member of a previous "cocktail". Any improvement in clinical response will be welcome, of course, but it would be scientifically inappropriate to draw the simple conclusion that the new drug was more effective *per se* than that which was replaced. Several alternative conclusions would be possible, not least that drug interaction alone was the important new mechanism with a minimum of improvement due to the single agent response.

It is fortunate that a "base-line" has been established in radiotherapy, involving the use of low LET ionising radiation delivered in a regime of fractionation, usually five daily doses per week over an average of four weeks up to a total dosage which is known to be tolerated by various normal tissues [25]. Innovations in radiotherapy can always be compared with such a regime which, while only based upon empirical studies, has provided a large body of clinical experience.

Empirical studies were the basis of modern radiotherapy, but these involved variations in time–dose relationships of *only one agent*. It is difficult to disentangle the many time and dosage variations for the new cancer drugs now being used to establish the basis of modern chemotherapy.

Briefly, these drugs can be classified as:

(1) Alkylating agents—busulphan, chlorambucil, cyclophosphamide, mechlorethamine (HN_2), melphalan, thiotepa and the nitrosoureas.

(2) Antimetabolites—cytosine arabinoside, fluorouracil (5-FU), mercaptopurine, methotrexate and thioguanine.

(3) Plant alkaloids—vinblastine and vincristine.

(4) Antibiotics—adriamycin, bleomycin, actinomycin D, daunorubicin.

(5) Other synthetic agents—dacarbazine (DTIC), hydroxyurea, procarbazine.

(6) Hormones—oestrogens, progesterones, testosterones and cortisones.

Each and every drug has been combined with radiation at some time but no attempt can be made to review the results of all such combinations, chiefly because it is doubtful whether enough valid data exist. Few enough data exist for the use of such drugs alone but Carter and Soper [24] have ranked those drugs which they do consider to have been adequately tested for activity against a number of different types of tumour. At the top of their list came 5-fluorouracil (5-FU) and methotrexate (MTX) with a 50 per cent success rate. 5-FU had been successful in 7 out of 14 types of tumour. This compares with 33 per cent (4 out of 12) for adriamycin and 25 per cent (2 out of 8) for bleomycin, the two newer antibiotics now undergoing active trial.

It is not proposed to discuss the interaction of radiation with hormones in this commentary because of the complications of this class of "drugs", which often occur as physiological humoral agents with effects which are even more difficult to predict [26] than those following treatment with all the other classes of drug.

BASIC MECHANISMS OF RADIATION

The biophysical mechanisms of radiation cell killing have been examined in great detail for cells that are proliferating, such as cultured cells [27]. It is only necessary to summarise those mechanisms directly relevant to drug–radiation interaction. Figure 3 shows a typical radiation dose–response curve for Chinese hamster ovary (CHO) cells in logarithmic culture. Survival of the colony-forming ability of single cells is seen to fall exponentially with increasing radiation dose. A large number of examples of such cell survival

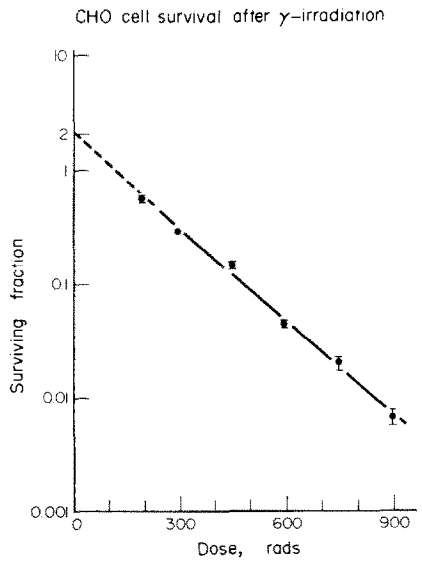

Fig. 3. Cell survival curve for CHO cells irradiated with ^{60}Co γ-rays.

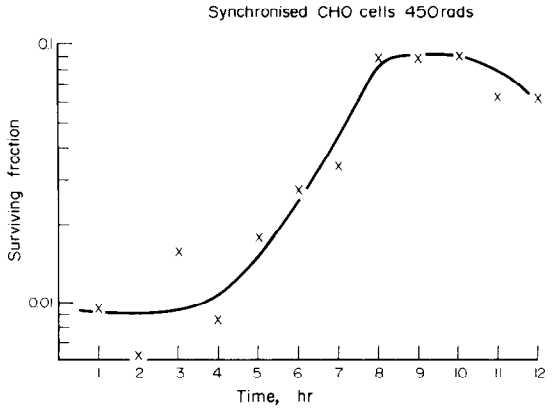

Fig. 4. Survival of synchronised CHO cells irradiated with 450 rads at different times after mitotic selection.

curves is now available [28], all with the same general characteristics. Consideration of the initial shape of such survival curves was the subject of the 6th L. H. Gray Conference [29] but the exponential portion of radiation cell survival curves conforms to a multi-target single hit model. The physical distribution of ionising radiation necessitates some such form of model compatible with random damage.

Figure 4 shows a plot of the survival of CHO cells which have been synchronised with respect to their progress through the cell cycle. Aliquots of such a synchronised cell population have then been irradiated with the same dose (450 rads). Survival is seen to be low at the beginning of the time scale when the cells had just completed mitosis and entered the G_1 phase of the cell cycle. As they progress through the cycle the cells show less sensitivity to this dose of radiation and by 9 hr ten times as many cells survive. This is at a point when DNA synthesis is now complete and the cells approach the G_2 phase of the cycle prior to mitosis at 12 hr.

Recovery mechanisms can nearly always be related to nucleic acid synthetic processes and these and other aspects of radiation biochemistry have been reviewed [28].

There remains just one aspect which is peculiar to radiation; namely G_2 block. It has already been shown that radiation is much more lethal when delivered to cells at the G_1–S transition point in the cell cycle (Fig. 4). G_2 block is a phenomenon wherein cells which have not necessarily absorbed a lethal dose of radiation exhibit delay in progress through the cell cycle, and this is maximal in G_2. The amount of cell cycle delay can be averaged out to an approximate value of 1 min per rad but the value rises to 1.4 min in G_2 compared with 0.4 minutes in G_1. This factor might be utilised in a drug–radiation combination where radiation is given first followed by a drug which is particularly active against cells in the G_2 phase, as reported for bleomycin [30]. Present regimes seem to ignore this rationale since the drug is administered before the radiation.

This is a particular example of a general rationale for drug–radiation interaction which combines one agent which slows down cell progress through a particular phase of the cell cycle (by means of a non-lethal effect) with a second agent that is maximally lethal in that same phase. The converse example to radiation then bleomycin in G_2, would be low dose methotrexate then radiation at the G_1–S transition [31].

DRUG MECHANISMS

The large amount of literature describing the mechanism of radiation is not matched by equivalent knowledge of the various drugs in common use. Dosimetry, for example, is a relatively very precise physical technique with radiation where the distribution in treated tissues can be precisely measured and the clinician can repeat a treatment in the confident knowledge that his prescribed dose will be faithfully delivered. For most drugs it is almost impossible to emulate this precision. Certain minimal information should nevertheless be available before a drug is used. What is the mode of action at the cellular level? Does the drug reach the intracellular target molecules by passive diffusion or active transport? Is the drug active in its original form or by some degrative product (e.g. after cyclisation in the case of nitrogen mustard and by liver metabolism in the case of cyclophosphamide)? What is the rate of degradation and loss of activity of the drug (e.g. the half-time of hydrolysis in water or preferably in some medium relevant to the *in vivo* milieu at 37°)? Does the drug degrade to a final product which is non-toxic? What are the pharmacokinetics? [4–8, 32].

Figure 5 shows an example of this latter point for the bifunctional alkylating agent methylene dimethane sulphonate (MDMS) for which the final product is formaldehyde! (Thus, the survival level never exceeds 40 per cent in Fig. 5.) Studies with this drug *in vitro* [33] were complicated by this fact which would be relatively unimportant *in vivo*. Since the half-life of hydrolysis of the drug *per se* is 22 min, the solution to the problem *in vitro* was to expose the cells to a 15-min treatment and then remove the residual drug from the culture medium. In this way the cytotoxic effect on the cells would be predominantly from the alkylating agent rather than formaldehyde.

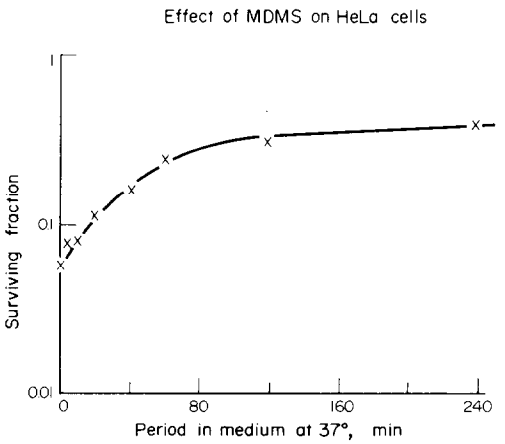

Fig. 5. Rate of inactivation of MDMS expressed in terms of increased survival of HeLa cells treated with aliquots of drug containing medium incubated at 37° (data of Nias and Fox [33])

Other drugs have much longer hydrolysis times in water but must be expected to be degraded more rapidly *in vivo*. *In vitro* studies need to be designed to use an appropriate period of drug treatment both for that reason, and also to take into account the extent of binding to cellular components. This proved to be an important consideration in the design of "split-dose" experiments with the monofunctional alkylating agent methyl methane sulphonate [34].

Solubility of the drug is another consideration. Is the drug soluble in aqueous medium and (apart from temperature effects) is its activity influenced by pH? Does any other change in the substrate influence drug activity? The platinum coordination complex *cis*-dichlorobis(cyclopentylamine) platinum II is insoluble in water and was therefore dissolved in dimethyl sulphoxide before dilutions were made in culture medium to produce the dose–response curve shown in Fig. 6. At the time of that study the effective half-life of the drug was 160 min [35]. A change in culture medium was then made and this included a small increase in the Cl^- ion concentration from 108 to 120 mM. This reduced the effective half-life of the drug to 45 min. The dose–response curve for a 1-hr exposure of cells reflected this: the D_0 rose from 9.3 μg/ml. shown in Fig. 6 to 14 μg/ml. This provides an example of competition between (a) reaction of drug molecules with proteins in its milieu and (b) its availability for transport and reaction with cellular components. The mechanism of action of each anti-cancer drug must involve interference with cell reproduction. Is this the result of an effect on nucleic acid templates, or enzyme substrates or what?

COMBINATION STUDIES

Cell and tissue cultures permit a wide variety of permutations and combinations of drugs with radiation—choice of drug, dosage of the two agents, time interval, choice of environment (the oxygen concentration, which is so important a factor in radiation sensitivity, may also influence the drug sensitivity of a cell) and other factors are all important considerations in the design of experiments *in vitro*. It is worth stressing, however, that it may often be more rewarding to choose some combination of factors which may prove relevant to *in vivo* and clinical situations. Thus, pharmacological evidence that the effective half-life of a drug is *n* min *in vivo* might lead to that period of time being chosen for the cells to be exposed to that drug *in vitro*. Radiobiologists certainly use the same radiation dosage regimes in the laboratory as are used in clinical practice.

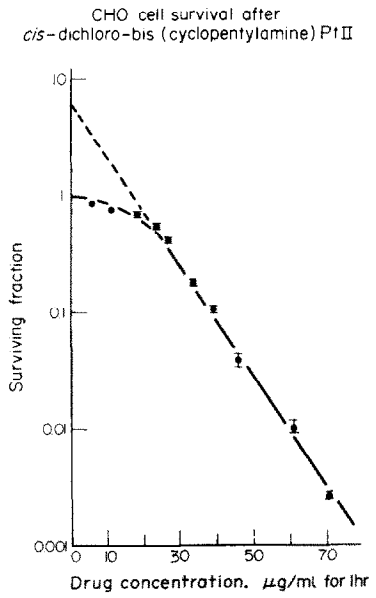

Fig. 6. Cell survival curve for CHO cells exposed to *cis*-dichloro-bis-(cyclopentylamine) Pt II.

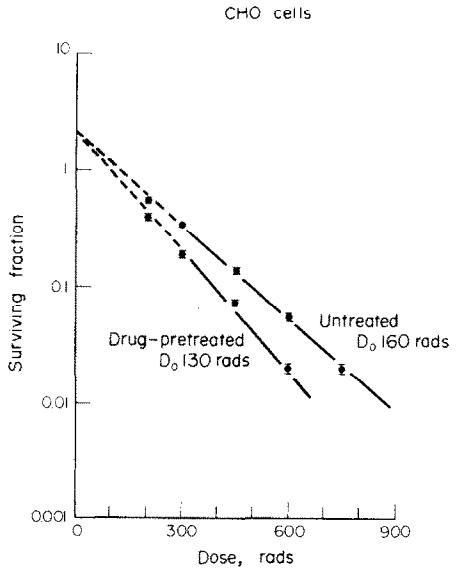

Fig. 7. Cell survival curves for CHO cells irradiated with ^{60}Co γ-rays with or without pretreatment with *cis*-dichloro-bis-(cyclopentylamine) Pt II– 26 μg/ml for 1 hr.

The experiment should then be designed to seek evidence for an effect of drug plus radiation which is *more* than additive. This might well be demonstrated by the sort of data shown in Fig. 7 for CHO cells treated either with radiation alone or radiation following treatment with the platinum coordination complex. Cell survival curves for both agents were shown earlier in Fig. 3 for radiation and Fig. 6 for the drug. Now the combination treatment is shown in this present figure to have a small but significant dose-modifying factor. After normalisation, the radiation survival curve of the drug-treated cells does *not* fit that of the cells treated by radiation alone— the radiation effect is greater. The combination is not just additive; there is potentiation by a factor of 1.23 in this case. Other drug–dosage combinations elicited a dose-modifying factor as high as 1.6 [36] and, with synchronised cells, the factor was found to be higher in the G_1 and late S phases of the cell cycle than in mid-S.

This is the sort of factor which is found with the new class of electron-affinic drugs, the nitroimidazoles [37–39]. These combine with hydrated electrons, the reducing member of the primary products of the radiolysis of water. This alters the redox potential in favour of the oxidative pathway and tends to sensitise

hypoxic cells to the effect of radiation. The most effective sensitising agent for hypoxic cells is oxygen itself, of course (by a factor in excess of 2.5), but oxygen diffusion to tumour cells is often inadequate due to the abnormal vascular supply in many tumours. The diffusion of a nitroimidazole drug is at least as good as that of oxygen, with the added advantage that while a relatively hypoxic cell will tend to reduce the local oxygen concentration by respiration, the concentration of the drug will remain at the level achieved by diffusion. The radiation chemical property of the electron-affinic drug will then be evident at the time of irradiation and dose modifying factors of 2.3 have been found which can be reproduced *in vivo* with C_3H tumours [38]. At an effective dose-level, however, nausea, vomiting and neurological symptoms occur. The present search is for drugs of this class which are less toxic.

REPEATED DOSES

It is up to the clinical scientist to design combination regimes based upon well established drug and radiation mechanisms. The designer should remember that single doses are the exception and that repeated treatments with combination regimes are the rule. The phenomenon of "recruitment" will therefore apply; this is a term used to designate the induction of a resting cell into the mitotic cycle or the induction of a slowly proliferating cell population to increase its rate of cell replication [40–41].

The result of such recruitment (and other inevitable consequences of repeated treatments with both drug and radiation) will be a tendency for both normal and tumour cell populations to cycle randomly. The search for cycle phase-specific drugs has produced some important fundamental observations [42] but unless a tumour cell population can be synchronised this sort of information cannot be utilised.

It is difficult enough to synchronise a mammalian cell population under laboratory conditions [43] and then only for one cycle, using physical methods *in vitro*. When it comes to drugs, the use of hydroxyurea *in vivo* has led to only partial synchrony [Ref. 44]. Perhaps more attention should be paid to normal circadian rhythms which may turn out to be sufficiently different from those of a particular tumour to provide some rationale for the optimal timing of drug–radiation therapy [45–46].

In conclusion, while the phenomena of recruitment of cells into cycle, natural circadian rhythms of cyclic activity and induced partial synchronisation of the cycle will all be important considerations in the mechanism of action of any drug–radiation combination, the opposites of all these phenomena will also be important. Thus, progression delay is the consequence of many drug treatments [47–48], as has already been discussed. The most common point of arrest in progression through the cell cycle by drugs is at the G_1–S boundary and this is also one of the most radiosensitive points in the cell cycle. While this remains the simplest rationale for drug–radiation interaction it must be remembered that an important aspect of progression delay is the relationship between the drug concentration and the degree of blockage of cells. The kinetics of this blockage need to be understood in relation to both the tumour and limiting normal cell populations. Since both radiation and drugs are usually administered in a fractionated schedule the optimum time interval between fractions must be allowed, for both the blockage and its subsequent release. The number of possible drug–radiation combinations is already large; the search for their optimum dose–time relationships is almost a forbidding task, but this should be the goal. The essential criterion of interaction must be some degree of potentiation (as in Fig. 7) of the effect of one cytotoxic agent by the other, and over a full range of dose levels.

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