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Original Paper

Paclitaxel as a Radiosensitiser: A Proposed Schedule of Administration Based on *In Vitro* Data and Pharmacokinetic Calculations

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Paclitaxel is efficacious against many human cancers. Because it blocks cells at the radiosensitive G2–M interface, paclitaxel has been investigated as a radiosensitiser. The results have been equivocal and somewhat contradictory. It is impossible to obtain proper pharmacokinetic calculations, aimed at obtaining maximum cytotoxicity and/or radiosensitisation, without knowing (i) how long the drug must be in contact with the cells, (ii) how long the effect lasts after the drug is removed from the cellular environment, (iii) whether the drug acts as a radiosensitiser even when, like *cis*-platinum, it is added after the radiation and (iv) what the minimum quantity of drug in the cellular environment is required for both chemotoxicity and radiosensitisation. The present work addresses the above questions. Two radioresistant cell lines of human origin were used, A375 melanoma and S549 lung carcinoma, in a clonogenic assay where only colonies with 50 or more cells were counted. For the irradiation, 6 MV X-rays were used. Any G2–M block was quantified by cell cycle kinetics analysis. From the results, a simulation of pharmacokinetics was conducted to calculate the schedule of administration of paclitaxel most likely to achieve and maintain significant chemotoxocity and radiosensitisation. The minimum concentration of paclitaxel for measurable cytotoxicity was 3 nM for both cell lines, but the drug was more toxic to the A549 cells. The minimum concentration for measurable radiosensitisation was 3 nM for A375 and approximately 0.1 nM for A549, but whereas above 3 nM the radiosensitivity increased in A375, it decreased above 1 nM for A549. A minimum of 18 h incubation with the drug was necessary for measurable effects and the radiosensitising effects were lost soon after its removal. There was no radiosensitisation if paclitaxel was added after the radiation, and, at the minimum effective concentrations, it caused only a minor and transient G2–M block. The pharmacokinetic calculations predict that 15 mg/m^2 paclitaxel given as a 1 h infusion 5 days/week for 3 weeks during the radiotherapy should achieve both cytotoxicity and radiosensitisation. The mechanism of radiosensitisation by paclitaxel at the concentrations suggested by our results does not appear to be via a G2–M block and is probably concentration dependent. The results imply that low-dose, daily infusions of paclitaxel for as long as possible during a course of radiotherapy are more likely to result in radiosensitisation and prolonged cytotoxicity than high-dose infusions given once a week.

Key words: paclitaxel, cytotoxicity, radiosensitisation, pharmacokinetics

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INTRODUCTION

THE TAXANES are a recently introduced class of antitumour agents which have been found to be active against a wide

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variety of human cancers. They bind to microtubules, thus inhibiting eukariotic cell replication and blocking cells in the G2 mitotic phase of the cell cycle [1, 2]. Because late G2 and M are known to be the most radiosensitive phases of the cell cycle, paclitaxel (Taxol®) has been extensively investigated as a radiosensitiser *in vitro* [3–8]. The results have shown that paclitaxel does indeed act as a radiosensitiser in many, but not all, cell lines studied and that, in general, the radiosensitising effect is accompanied by a substantial G2–M block.

Based on the *in vitro* results and extensive pharmacokinetic studies in humans [9–11], Choy and associates [12] undertook a phase I clinical trial of combined modality treatment (CMT) of advanced stage non-small cell lung cancer (NSCLC). They recently reported that the combination of paclitaxel and fractionated radiotherapy gave promising results, using the maximum tolerated dose of 60 mg/m^2 of paclitaxel given as a 3 h infusion once a week throughout a 6 week course of fractionated radiotherapy [13]. Previous attempts at CMT (radiotherapy + *cis*-platinum) of this intractable disease have given equivocal results [14, 15] and new, promising approaches are to be welcomed.

The equivocal or negative results obtained in previous clinical trails using CMT for advanced, non-operable NSCLC [14, 16] led us to explore the possibility of using concurrent paclitaxel and fractionated radiotherapy. Paclitaxel by itself has been shown to be an active first-line agent against NSCLC [17–19] so that, even if there is no interaction but merely additivity between the two modalities, the combination might still yield positive results. Before starting such a study, it is necessary to decide how to administer the two modalities so as to have the best likelihood of achieving additivity or, preferably supra-additivity, i.e. radiosensitisation. Although much mechanistic data is available from the literature, the answers to certain crucial questions are still lacking. For example, it is known that radiosensitisation is seen only if the drug has been in contact with the cells for 12–24 h, but the duration of the radiosensitising effect after removal of the drug is not known (although Steren and associates [5] seem to suggest that a 90 min incubation with paclitaxel prior to irradiation was sufficient to cause radiosensitisation and a long-lasting G2–M block). Neither is it known if paclitaxel, like some other drugs (e.g. *cis*-platinum), shows a radiosensitising effect even when added to cell cultures after irradiation [20]. Since paclitaxel is cleared relatively quickly from the circulation [10], it is essential to know the answers to the above questions in order to calculate a realistic administration schedule—a schedule which is likely to achieve and maintain the minimum tissue concentration of paclitaxel required for both cytotoxicity and radiosensitisation.

Milas and colleagues [25] have recently reported the results of *in vivo* experiments in tumour-bearing mice using a combination of paclitaxel and radiation. Their results indicate that *in vivo* paclitaxel radiosensitises tumour cells via an indirect route involving cell death by apoptosis, followed by cell loss and reoxygenation. These results, although very relevant for the understanding of the mode of action of paclitaxel, are not useful in the context of the present investigation because (a) they used doses of paclitaxel which are unusable clinically (40–60 mg/kg, equivalent to 2.8–4.2 g of the drug to a 70 kg patient!) and (b) they used single doses of radiation of 1500–8200 cGy—again well out of the range of radiation doses in current clinical use. One useful point in the paper by Milas and colleagues is that, as has been repeatedly found by many other investigators *in vitro*, maximum radiosensitisation is not seen *in vivo* for many hours following injection of the drug (24–48 h).

With this in mind, and the need to know what safe injection schedule of paclitaxel is most likely to result in radiosensitisation throughout a standard course of fractionated radiotherapy, *in vitro* studies were conducted which sought to answer the following questions: (i) what is the actual shape of the cell survival curves against paclitaxel concentration and are they the same for cell lines?; (ii) what are the minimum concentrations of drug in the culture medium required for achieving measurable cytotoxicity and radiosensitisation?; (iii) what is the minimum duration of contact with paclitaxel that the cells required to produce measurable radiosensitisation and is this the same for all cell lines?; (iv) how long does the radiosensitisation effect last after the drug is removed from the culture medium?; (v) does paclitaxel produce radiosensitisation when added to the culture medium after irradiation of the cells?; and (vi) at the minimum concentration required for radiosensitisation, does paclitaxel produce a significant G2–M block?

Based on our *in vitro* studies and on published plasma clearance curves for paclitaxel in humans, a schedule of administration was calculated which would achieve and maintain plasma levels of the drug at, at least, three times the minimum required for radiosensitisation—that is, the concentration at any time at the tissue level was assumed to be at least three times lower than that in the plasma.

MATERIALS AND METHODS

Cells and cell culture

Two cell lines were used: A375 derived from a human melanoma and A549 derived from a human lung carcinoma. The A375 cells were maintained in plastic culture bottles in RPMI 1640 medium (Flow, Irvine, U.K.) supplemented with 10% fetal calf serum (FCS) (Sera Laboratory, Grawley Down, U.K.) and gentamicin. The A549 cells were grown in Eagle's minimal essential medium (EMEM) (Flow) supplemented with FCS, non-essential amino acids, vitamins and gentamicin. All cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Cells were plated in 35 mm culture dishes with 2 ml of culture medium and allowed to attach for 24 h. The number of cells per dish was chosen so that on average 50 colonies would survive after treatment. Paclitaxel, dissolved in a small quantity of ethanol immediately before use and diluted with culture medium, was then added at various molar concentrations and the cells incubated for various times up to 48 h in the presence of the drug. The cells were given 400 cGy irradiation using a Varian 6 MV linear accelerator (Varian S.p.a., Torino, Italy).

It may be argued that in order to obtain accurate radiosensitising factors more than one radiation dose (preferably full survival curves) should be used. However, the purpose of the present investigation was simply to produce the necessary data for the pharmacokinetic calculations, and for this purpose we felt that comparing results at one radiation dose would be sufficient. Following irradiation, the cells were then immediately washed free of the drug and 2 ml of drug-free medium was added. Non-irradiated controls were

Table 1. Clonogenic cell survival after 24 h incubation with various concentrations of paclitaxel only and paclitaxel + 400 cGy irradiation

Paclitaxel (nM)	% Survival (mean ± S.D.)* A375 Paclitaxel only	A375 Paclitaxel + 400 cGy†	A549 Paclitaxel only	A549 Paclitaxel + 400 cGy†
0.05	98.1 ± 3.7	not done	98.0 ± 3.1	not done
0.10	99.0 ± 5.7	98.7 ± 4.8	98.0 ± 4.2	79.3 ± 3.3
0.50	97.3 ± 2.2	86.7 ± 2.7	97.1 ± 2.2	74.0 ± 2.1
1.0	96.7 ± 4.1	78.2 ± 3.3	98.1 ± 3.9	70.1 ± 1.9
2.0	78.0 ± 2.6	not done	50.0 ± 1.8	not done
3.0	60.1 ± 1.9	16.8 ± 0.7	24.1 ± 4.7	9.8 ± 1.1
5.0	42.3 ± 1.6	2.0	21.5 ± 2.1	1.8
10.0	28.0 ± 4.7	0.6	10.3 ± 1.2	0

*The values represent the mean of six dishes. †Corrected for the effects of radiation only.

treated similarly. Three dishes were used for each drug concentration and irradiation dose, and each experiment was repeated twice giving a total of six dishes per point. The cells were incubated for 7 days after which the colonies with more than 50 cells were counted. In some experiments, the same procedures were carried out using *cis*-platinum at a concentration of 1 μg/ml [30].

Flow cytometry

Cells cultured as described above and exposed to various concentrations of paclitaxel for a minimum of 24 h were harvested from proliferating monolayers by trypsinisation and diluted in growth medium to a density of 2×10^6 cells/ml. They were then fixed in nine parts of 70% ethanol to one part of Hank's balanced salt solution (Sigma Chimica, Milan, Italy) and stored overnight at 4°C. The cells were then lysed using a 9:1 mixture of 0.05 M sodium phosphate/25 mM citric acid to which 0.1% Triton-X-100 (Sigma Chimica) was added, and brought to pH 7.8. To each 0.5 ml of each cell suspension was added 0.5 ml of a 100 μg/ml solution of propidium iodide (Coulter Immunology, Hialeah, Florida, U.S.A.) and the mixture incubated at room temperature for 30 min. Chicken erythrocytes (Coulter Immunology) were similarly treated and served as DNA standards.

The cell samples were analysed in a Coulter EPICS flow cytometer (Coulter, Epics Division, Hialeah, Florida, U.S.A.) run in manual mode. Some 20 000 events were routinely collected. Single-parameter DNA histograms from the gated list mode were analysed for cell cycle distribution.

Pharmacokinetic simulation

We sought a schedule of paclitaxel administration which would achieve: (a) a plasma concentration always above the effective concentration 20 h before radiotherapy and during the radiotherapy sessions; (b) plasma concentrations which, according to published data, would not lead to severe toxic reactions; (c) a minimum plasma concentration at least three times the *in vitro* radiosensitising concentration (3 nM, but see Results); and (d) a treatment schedule which could be implemented in an outpatient clinic (eg. short daily intravenous (i.v.) infusions).

For the simulation, it was assumed that paclitaxel followed first-order kinetics since at low doses no saturation of the clearance mechanism has been reported. The calculations of average steady-state concentrations (C_{ss}) were carried out using non-compartmental equations after Gibaldi and Perrier [21]. Average estimates of total body clearance (TBCl = 394 ml/min/m^2) and elimination half-life ($T\frac{1}{2}$ = 4.9 h) were taken from the data published by Rowinsky and associates [10] and average peak concentrations after a single 15 mg/m^2 60 min i.v. infusion were taken from the work of Grem and associates [22]. Minimum concentrations after the first dose and at steady state (C_{ss}) were calculated using a non-linear curve-fitting program.

RESULTS

Table 1 shows both cytotoxicity and radiosensitisation data on cell survival following incubation for 24 h with various concentrations of paclitaxel and paclitaxel plus 400 cGy radiation (corrected for the effects of the radiation alone). The A549 cell line appears to be somewhat more sensitive to the drug than A375.

Below 2 nM, paclitaxel had minimal measurable toxicity for either cell line. Paclitaxel radiosensitised both cell lines, but whereas an effect was seen only at concentrations of 3 nM or greater in A375, in the lung adenocarcinoma line, A549, it seems to show some degree of radiosensitisation even at non-cytotoxic concentrations as low as 0.1 nM. At concentrations greater than 3 nM, the clonogenic survival in the paclitaxel + 400 cGy-treated A549 cells was too low for accurate measurement. It has been known for some time that a period of incubation of 12–20 h is required for radiosensitisation [3, 6, 7] and the data in Table 2 show that this was also true for the cell lines used in this study and under our culture conditions. Although the A549 cells were radiosensitised by very low concentrations of paclitaxel, Figure 1

Table 2. The effect of incubation time with 3 nM paclitaxel followed immediately by 400 cGy irradiation on the degree of radiosensitisation for both cell lines used

Hours of incubation with 3 nM paclitaxel before 400 cGy RT	Radiosensitisation factor* A375	A549
4	1.06	1.21
12	1.18	1.68
21	2.30	2.19
24	3.21	2.68

*Calculated from the fractional survival after correction for the effects of paclitaxel alone and radiation alone. The values represent the mean of six dishes.

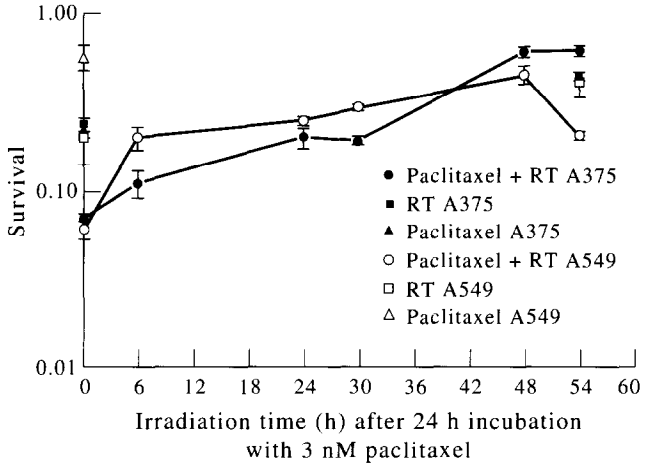

Figure 1. Duration of radiosensitising effect after the removal of paclitaxel from the culture medium (24 h incubation). Solid symbols: A375 cells; open symbols: A549 cell line. The cells were incubated with 3 nM paclitaxel for 24 h and then washed and resuspended in fresh medium. They were then given 400 cGy irradiation (RT) either immediately or at the times after washing shown on the abscissa. The symbols at zero time show the survival of cells incubated with paclitaxel only (▼, ▽) or given 400 cGy irradiation without previous paclitaxel treatment (■, □). The irradiation only experiments were carried out at 0 and 54 h. Three experiments were carried out for each point and the error bars represent one standard deviation.

shows that the effect is lost very quickly in this cell line after removal of the drug from the culture medium. The melanoma cells seemed to maintain some degree of sensitisation for 8–10 h after the removal of paclitaxel.

Figure 2 shows that for the A549 cell line, as expected, *cis*-platinum radiosensitised cells even when added to the culture medium a few hours after the radiation, whereas paclitaxel did not. The radiosensitising factor at 400 cGy

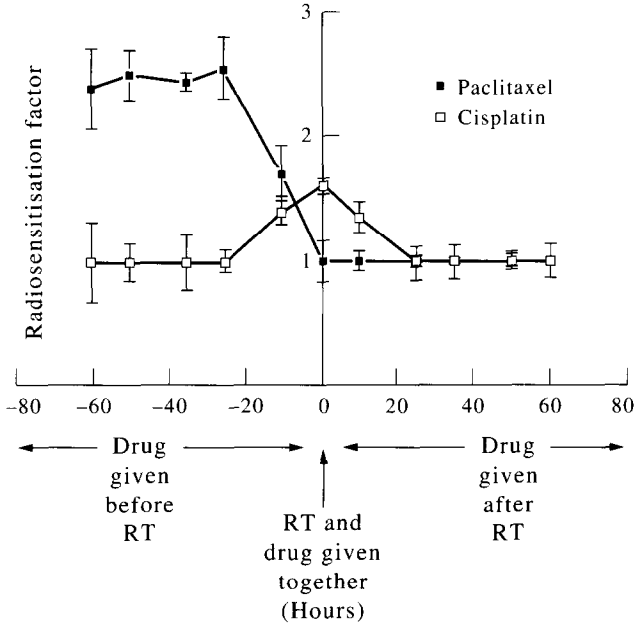

Figure 2. Radiosensitising effect of 3 nM paclitaxel or 1 μg/ml *cis*-platinum on A549 cells as a function of the time between the addition of the drug and irradiation (RT). The symbols represent the mean ± S.D. of three separate experiments.

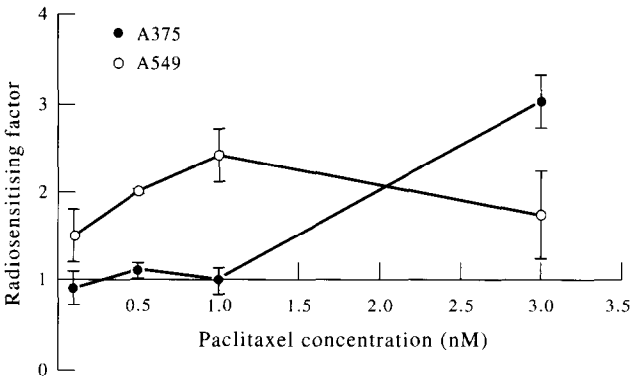

Figure 3. Radiosensitising factor as a function of paclitaxel concentration for both cell lines used. The symbols represent the mean ± S.D. of three separate experiments.

for both cell lines are plotted against paclitaxel concentration in Figure 3. Radiosensitisation increased steeply between 1 nM and 3 nM for the A375 cell line, but decreased above 1 nM in the A549 cells. This might explain the discrepancy between the present results and those of Liebman and associates [3, 4], who used concentrations of paclitaxel of $10–10^4$ nM.

The cytofluorimetry results are shown in Tables 3 and 4. As can be seen, they failed to show major accumulation of cells at the G2–M interface after 24 h incubation, even at a paclitaxel concentration of 10 nM which produced a considerable degree of radiosensitisation in the A375 cells.

Figure 4 shows the plasma concentration versus time curve obtained by simulation of a patient with (averaged) paclitaxel pharmacokinetics receiving a daily 60 min i.v. infusion of 15 mg/m^2 paclitaxel for 5 consecutive days. After the first infusion, C_{max} would be 310 nM and C_{min} would be 14 nM. With the subsequent doses, a small accumulation in the plasma concentration would be observed, leading to a steady-state concentration of 32 nM, a C_{min} of 17 nM and a C_{max} of 345 nM. Steady state would be achieved after the second dose.

Large individual variations in paclitaxel pharmacokinetics have been reported (TBCl = 394 ± 203 ml/min/m^2). With our proposed schedule, only 2.5% of patients should have TBCl above 800 ml/min/m^2 (mean ± 2 S.D.) and therefore would have a C_{min} below the target plasma concentration of

Table 3. Cell cycle distribution of A549 lung cancer cells after exposure to paclitaxel (single experiment)

Time	Paclitaxel (nM)	% in cell cycle GI	S	G2–M
0	0	76.2	13.1	10.7
12	1	38.6	47	14.3
	3	53.8	36.4	9.8
	10	64.7	27.3	8
18	1	41.8	44.3	13.9
	3	41.6	35.7	22.7
	10	43.3	47.2	9.5
21	1	58.7	30.2	11.8
	3	52.7	36.1	11.2
	10	39.8	40.8	19.4
24	1	62	29.3	8.7
	3	52.8	40.7	6.5
	10	39.6	45.5	14.9

Table 4. Cell cycle distribution of A375 malignant melanoma cells after exposure to paclitaxel (single experiment)

Time	Paclitaxel (nM)	% in cell cycle GI	S	G2–M
0	0	57.9	22.2	9.9
9	1	23.9	47.4	28.7
	3	57.9	32.2	9.9
	10	22.7	53.4	23.9
12	1	34.8	59.6	5.6
	3	36.8	58.2	5.0
	10	41.8	51.8	6.3
18	1	32.4	59.1	8.6
	3	42.0	56.3	1.7
	10	35.4	58.9	5.7
21	1	33.4	58.8	7.8
	3	36.9	59.3	3.8
	10	48.9	46.0	5.1
24	1	35.2	56.6	8.2
	3	30.9	52.5	16.6
	10	46.8	34.2	19.0

9 nM. Using the same method of calculation, it can be shown that, for infusions of 10 mg/m^2, 30% of patients would have a C_{min} below 9 nM, whereas with 12 mg/m^2, 16% of the patients would still be below our target concentration. For comparison with the work of Choy and associates [12], the results of a simulation of a patient, again with averaged pharmacokinetics, given one 3 h infusion of 75 mg/m^2 given once a week are also shown in Figure 4. Although the total dose and systemic exposure (AUC) are the same for both schedules, the time above the target concentration could be 40 h in the once a week treatment and 120 h in the daily, 5 times a week option.

DISCUSSION

It has been known that paclitaxel radiosensitises a variety of cells *in vitro* [3–8], and recently Milas and associates have shown that it is also a potent radiosensitiser *in vivo* [23–25]. Paclitaxel appears to radiosensitise cells through more than one mechanism: a block at the radiosensitive G2–M interface, apoptosis and reoxygenation. The latter mechanism, recently reported to occur *in vivo* by Milas and associates [23], is obviously of major importance in concurrent chemotherapy/radiotherapy. In this context, our results show that the highly radioresistant melanoma cell line A375 was also sensitised (at least *in vitro*) by paclitaxel, albeit at higher concentrations of the drug. This could be a reflection of the ease with which paclitaxel is able to cross the membrane of different cells, or of the duration of its intracellular retention.

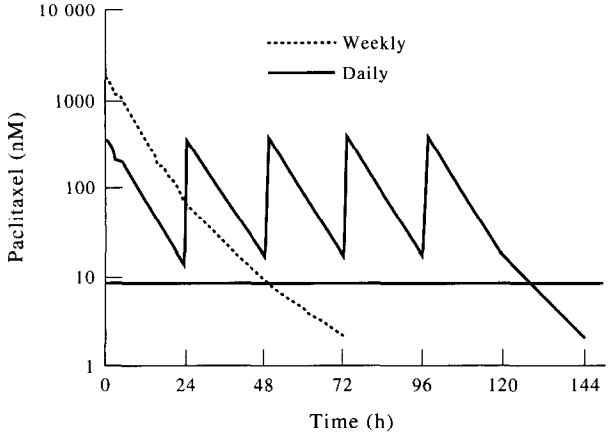

Figure 4. Computer simulation of paclitaxel plasma concentration versus time for two administration schedules: Solid line = 15 mg/m^2/1 h infusion once a day 5 days a week. Broken line = 75 mg/m^2/3 h infusion once a week. The solid horizontal line shows the target concentration of 9 nM.

Some of the published work (e.g. [23]) seems to suggest that paclitaxel may radiosensitise via a complex mechanism such as apoptosis → cell depopulation → reoxygenation. This would mean that paclitaxel need not be present in the cellular environment at or near the time of irradiation. However, our results, as well as those of many other authors [3, 6, 7], show that, at the radiation dose used, maximum radiosensitisation is not achieved unless the drug has been in contact with the cells for approximately 24 h (Figure 3) and that the radiosensitising effect is lost very quickly when, after 24 h incubation, the cells are washed prior to irradiation (Figure 1). In our opinion this mediates against an indirect mechanism of radiosensitisation by paclitaxel. The length of incubation required for radiosensitisation (>20 h) suggests rather that paclitaxel may have to be taken up and retained intracellularly in order for it to exert its radiosensitising effect. The mechanism(s) by which paclitaxel produces its cytotoxic and radiosensitising effects has by no means been satisfactorily elucidated and further experimentation is required.

Our data (degree of radiosensitisation and length of incubation) are comparable with most of the published data. However, in contrast with the work of Steren and associates [5], who found a substantial and long-lasting G2–M block in a human ovarian cell line after only 90 min incubation with 5 nM paclitaxel followed by irradiation 48 h later, neither of our cell lines showed a significant G2–M block. The explanation for this discrepancy may be due to the use of different cell lines since we have shown that both the cell lines in our study also differed in their response to paclitaxel and to paclitaxel + irradiation.

Our data are also in disagreement with the experimental results reported recently by Hennequin and his colleagues [29]. Their data showed that paclitaxel, as well as the other semisynthetic taxane, docetaxel, can act either as radiosensitisers or radioprotectors depending on various factors such as radiation dose, cell culture density etc. Their IC_{50} for paclitaxel was 225 nM. We did not determine the IC_{50}, but from Table 1, our IC_{50} appears to be in the region of 4 nM and at concentrations greater than 30 nM we had 100% cell kill. Furthermore, Hennequin and associates reported both radiosensitising and radioprotective effects after only 1 h total incubation with the drugs (30 min before and 30 min after 500 cGy irradiation). We were unable to show any effect of the combined treatment for pre-incubation periods shorter than around 12 h (Figure 3) even though, in the combined modality experiments, we used a similar radiation dose (400 versus 500 cGy). One possible explanation for the widely different results may be that the *in vitro* effects of the taxanes are much more dependent on such factors as cell type, cell density and culture conditions than previously thought. It should also be noted that, whereas we prepared our paclitaxel solution immediately prior to use, Hennequin and associates used ethanol solutions which had been stored at −20°C for unspecified periods of time. In preliminary ex-

periments (results to be published), we found, using HPLC methodology, that after 2 months storage in ethanol at −25°C, paclitaxel appears to break down producing an HPLC peak with very short retention time, presumably more water soluble, and corresponding to 40–50% of the total quantity of drug.

Whatever its mode of action, in the *in vivo* situation, the drug must sooner or later come into contact with the relevant cells in sufficient quantities in order to exert its effect(s). The results presented in this paper suggest that the administration schedule chosen by Choy and associates [12], consisting of one high-dose infusion once a week during a protracted course of fractionated radiotherapy, may not achieve the best results (Milas and associates [23, 24] achieved very high radiosensitisation factors in their animal model using high, non-clinical single doses of radiation). This is because such a schedule will almost certainly not maintain minimum radiosensitising, or for that matter cytotoxic, plasma levels of approximately 9 nM throughout the course of treatment. Furthermore, since the bulk of experimental evidence suggests that, in order to obtain measurable radiosensitisation, the drug must be in contact with the cells for some 12–20 h, the once-a-week schedule is most probably effective for only a few hours a week. This follows from the data in Figures 1 and 2 which show that: (a) paclitaxel becomes ineffective as a radiosensitiser very soon after it is removed (or falls below a critical concentration) from the cellular environment; (b) paclitaxel must have been present for some time before the irradiation in order to exert its maximum radiosensitising effect; and (c) the presence of even high doses of paclitaxel after irradiation does not lead to radiosensitisation. It is difficult to see how a rational CMT protocol can be arrived at without taking into account these crucial facts. Lower daily doses, as we suggest, have not only a better chance of achieving and maintaining the required tissue levels, but could also be less toxic.

The choice of plasma levels at least three times the tissue levels required for cytotoxicity and radiosensitisation in the least sensitive cell line (A375) is critical to the whole argument. This factor of three was adopted after a review of the few published data on the ratios of plasma to tumour concentration for a variety of drugs and tumours of different histologies [26–28]. The available data show that a plasma to tumour tissue ratio of three should not be far from the truth—allowing of course that there will be almost certainly substantial variations not only between tumours of different histology but also between individuals with the same type of tumour.

Our proposed schedule, therefore, consists of 15 mg/m^2 as a 1 h infusion, once a day, 5 days a week for 3 weeks. The total quantity of paclitaxel (225 mg/m^2) should be well tolerated according both to the published data [12, 13, 17] and our experience with paclitaxel in the treatment of ovarian carcinoma. Furthermore, the above schedule does not impose a great strain either on the staff or the patient and, if it should prove to be well tolerated, could be extended to more than 3 weeks and thus hopefully increase the duration of the radiosensitising effect to practically the whole of the course of radiotherapy.

A major problem could be the acute toxicity which may necessitate the repeated use of dexamethasone or similar drugs. We have been unable to find any data in the literature regarding the acute toxic effects of fast daily infusions of low-dose paclitaxel, and a phase I clinical trial will, therefore, be necessary in order to assess the possible duration and safety of our proposed scheme. Such a trial is currently being organised in our department.

1. Huizing MT, Sewberath Misser VH, *et al.* Taxanes: a new class of antitumor agents. *Cancer Invest* 1995, **13**, 381–404.
2. Guenard D, Guéritte-Vogelein F, Dubois J, Poitier P. Structure–activity relationships of taxol and taxotere analogues. *Monogr Natl Cancer Inst* 1993, **15**, 79–82.
3. Liebmann J, Cook JA, Fisher J, Teague D, Mitchell JB. Changes in radiation survival curve parameters in human tumor and rodent cells exposed to Taxol (paclitaxel^{R}). *Int J Radiation Oncol Biol Phys* 1994, **29**, 559–564.
4. Liebmann J, Cook JA, Fisher J, Teague D, Mitchell JB. *In vitro* studies of taxol as a radiosensitizer in human tumor cells. *J Natl Cancer Inst* 1994, **86**, 441–446.
5. Steren A, Sevin B-U, Perras J, *et al.* Taxol as a radiation sensitiser: a flow cytometric study. *Gynecol Oncol* 1993, **50**, 89–93.
6. Tishler RB, Schiff PB, Geard CR, Hall EJ. Taxol: a novel radiation sensitizer. *Int J Radiat Oncol Biol Phys* 1992, **22**, 613–617.
7. Minarik L, Hall EJ. Taxol in combination with acute and low dose rate irradiation. *Radiother Oncol* 1994, **32**, 124–128.
8. Stromberg JS, Lee YJ, Armour EP, Martinez AA, Corry PM. Lack of radiosensitization after paclitaxel treatment of three human carcinoma cell lines. *Cancer* 1995, **75**, 2262–2268.
9. Wiernik PH, Schwartz EL, Strauman JJ, *et al.* Phase I clinical and pharmacokinetic study of taxol. *Cancer Res* 1987, **47**, 2486–2493.
10. Rowinsky EK. Clinical pharmacology of taxol. *Monogr Natl Cancer Inst* 1993, **15**, 25–37.
11. Beijnen JH, Huizing MT, ten Bokkel Huinink WW, *et al.* Bioanalysis, pharmacokinetics, and pharmacodynamics of the novel anticancer drug paclitaxel (taxol). *Semin Oncol* 1994, **21** (Suppl 8), 53–62.
12. Choy H, Akerley W, Safran H, *et al.* Phase I trial of outpatient weekly paclitaxel and concurrent radiation therapy for advanced non-small-cell lung cancer. *J Clin Oncol* 1994, **12**, 2682–2686.
13. Akerley W, Choy H. Concurrent paclitaxel and thoracic radiation for advanced non-small cell lung cancer. *Lung Cancer* 1995, **12** (Suppl 2), S107–S115.
14. Trovò MG, Minatel E, Franchin G, *et al.* Radiotherapy versus radiotherapy enhanced by cisplatin in stage III non-small cell lung cancer. *Int J Radiat Oncol Biol Phys* 1992, **24**, 11–15.
15. Schaake-Koning C, van den Bogaert W, Dalesio O, *et al.* Effects of concomitant cisplatin and radiotherapy on inoperable non-small cell lung cancer. *N Engl J Med* 1992, **326**, 524–530.
16. Mirimanoff RO. Concurrent chemotherapy (CT) and radiotherapy (RT) in locally advanced non-small cell lung cancer (NSCLC): a review. *Lung Cancer* 1994, **11** (Suppl 3), S79–S99.
17. Murphy WK, Fonssella FV, Winn RJ, *et al.* Phase II study of taxol in patients with untreated advanced non-small cell lung cancer. *J Natl Cancer Inst* 1993, **85**, 384–394.
18. Ettinger DS. Taxol in the treatment of lung cancer. *Monogr Natl Cancer Inst* 1993, **15**, 177–179.
19. Comis RL, Friedland DM. New chemotherapy agents in the treatment of advanced non-small cell lung cancer: an update including data from the Seventh World Conference on Lung Cancer. *Lung Cancer* 1995, **12** (Suppl 2), S63–S99.
20. Pearson AE, Steel GG. Chemotherapy in combination with pelvic irradiation: a time dependence study in mice. *Radiother Oncol* 1984, **2**, 49–55.
21. Gibaldi M, Perrier D. *Non-Compartmental Analysis Based on Statistical Moment Theory in Pharmacokinetics*, 2nd edn. Marcel Dekker Inc., New York, 1982, 409–441.
22. Grem JL, Tutsch KD, Simon KJ, *et al.* Taxol administered as a short IV infusion daily for 5 days. *Cancer Treat Rep* 1987, **71**, 1179–1180.
23. Milas L, Hunter NR, Mason KA, Milross CG, Saito Y, Peters LJ. Role of reoxygenation in induction of enhancement of tumor radioresponse by paclitaxel. *Cancer Res* 1995, **55**, 3564–3568.

24. Milas L, Hunter NR, Kurdoglu B, *et al.* Kinetics of mitotic arrest and apoptosis in murine mammary and ovarian tumors treated with taxol. *Cancer Chemother Pharmacol* 1995, **35**, 297–303.
25. Milas L, Hunter NR, Mason KA, Kurdoglu B, Peters LJ. Enhancement of tumor radioresponse of a murine mammary carcinoma by paclitaxel. *Cancer Res* 1994, **54**, 3506–3510.
26. Donelli MG, D'Incalci M, Garattini S. Pharmacokinetic studies of anticancer drugs in tumour-bearing animals. *Cancer Treat Rep* 1984, **68**, 381–400.
27. Litterst C, LeRoy AF, Guarino AM. Disposition and distribution of platinum following parenteral administration of *cis*-diamminedichloroplatinum(II) to animals. *Cancer Treat Rep* 1979, **63**, 1485–1492.
28. Zanelli GD, Rota L, Trovò MG, *et al.* The uptake of ^{3}H-vincristine by a mouse carcinoma during a course of fractionated radiotherapy. *Br J Cancer* 1989, **60**, 310–314.
29. Hennequin C, Giocanti N, Favaudon V. Interaction of ionizing radiation with Paclitaxel (Taxol) and Docetaxol (Taxotere) in HeLa and SQ20B cells. *Cancer Res* 1996, **56**, 1842–1850.
30. Begg AC, Van Der Kolk PJ, Dewit L, Bartelink H. Radiosensitization by cisplatin of RIF1 tumour cells *in vitro*. *Int J Radiat Biol* 1986, **50**, 871–884.