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Lack of Therapeutic Gain When Low Dose Rate Interstitial Radiotherapy is combined with Cisplatin in an Animal Tumour Model

Lucas Pop, Peter Levendag, Cock van Geel, Inge-Karina Deurloo and Andrie Visser

The interaction of cisplatin with low dose rate interstitial radiotherapy was studied in an animal tumour model with a range of dose rates commonly used in clinical low dose rate brachytherapy. Small pieces of R1-rhabdomyosarcoma were implanted subcutaneously in the flanks of female Wag/Rij rats. When the tumour had grown to the desired treatment volume, four afterloading catheters were inserted in the tumour in a square geometry, and a fixed spacing was attained by means of a template. Subsequently, four 2 cm Ir^{192} wires were inserted. A range of tumour doses of 20–120 Gy at a mean dose rate of 48 cGy/h was applied; 15 mins before the implant an intraperitoneal bolus injection of 3 mg/kg cisplatin was given. For growth delay and cure rate, no modification of the effects of low dose rate brachytherapy by the addition of cisplatin was observed. The observed effects of the combination of cisplatin with low dose rate interstitial radiation in relation to the animal tumour are discussed.
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INTRODUCTION

THE INTERACTION of cisplatin with high dose rate irradiation has been studied extensively on tumour and normal tissues, both *in vivo* and *in vitro*. A varying degree of enhancement of the radiation effect by cisplatin was observed *in vitro* and several mechanisms have been proposed to explain this, such as a true radiosensitising effect [1–3] and inhibition of sublethal and potential lethal damage repair [1,4,5]. These effects were sometimes more pronounced in the absence of oxygen [4,5] while occasionally just the opposite has been reported [5–7]. *In vivo*, different dose enhancement factors have been reported in tumours and normal tissues. Depending on dose, timing and sequence a maximum dose enhancement factor was observed when the highest dose of an intraperitoneal bolus injection of cisplatin was given just before the start of the irradiation or with fractionated drug and radiation treatment [8–11].

The inhibition of sublethal and potential lethal damage repair *in vitro*, the diminshed recovery in tumours in split dose experiments *in vivo* [12] and the supra-additive effects obtained *in vivo* after fractionated schedules of both the drug and radiation treatment in two animal tumour models [10,11,13,14] were of particular interest to the design of the present study. It was argued that, if the modification of the radiation effects by cisplatin is really based on inhibition of sublethal damage repair,

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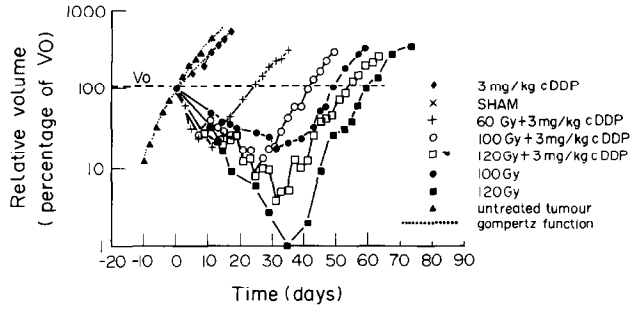

Fig. 1. Tumour volume relative to the volume at the start of the treatment at day 0 (=V_0) for a range of doses of low dose rate interstitial radiotherapy alone (LDR-IRT) or combined with an intraperitoneal bolus injection of 3 mg/kg cisplatin. The growth curve of an untreated tumour is also shown, fitted by a Gompertz function.

the combination of cisplatin with low dose rate interstitial radiation therapy (LDR-IRT) could lead to a strong amplification of the radiation effect.

The plan of investigation for the present study was to compare quantitatively the anti-tumour effects of LDR-IRT alone with LDR-IRT + cisplatin in an animal tumour model, in which the IRT was applied by interstitially implanted radioactive sources with a range of dose rates very similar to that used in the low dose rate brachytherapy clinical practice.

MATERIALS AND METHODS

Experimental tumour system

The rat rhabdomyosarcoma (R1), growing subcutaneously in the flank of WAG/Rij female rats was used as an animal tumour model. Details about origin, growth characteristics and radiosensitivity have been published before [15–17]. Serial passage of the tumour was accomplished by transfer of small pieces of tumour of about 1–2 μl subcutaneously in the left flank of 15–20-week-old WAG/Rij female rats. The mean growth curve of 6 untreated tumours (Fig. 1) could be fitted by a Gompertz function, e.g. $V(t)=V_{0.exp}\{a/k).(1-exp^{-kt})\}$, yielding $a = 0.25\ d^{-1}$ and $K = 0.045\ d^{-1}$. This means that at the start of the treatment at day 0 (at a median time of 13 days after implantation) tumour volume doubling time amounts to $\ln 2/a = 2.77$ days, but growth rate decreases exponentially thereafter with a half time of $\ln 2/k = 15.4$ days.

Irradiation technique

When the tumours had grown to the desired treatment volume of 1750–2500 μl (median tumour volume at the start of the treatment, V_0, was 1900 μl), four afterloading catheters were implanted in the tumour in a square geometry with a fixed spacing of 7 mm by means of a template. Using the template, four ^{192}Ir wires of 2 cm each with a mean activity of 4.6×10^7 Bq/cm (range $3.5–5.9 \times 10^7$) were implanted in the tumour. Isodose profiles were generated by computer planning as has been described before [18]. The irradiation time for a minimum tumour dose was computed from these data by estimating from isodose plots and dose rate calculations in a range of points the first enveloping isodose around the tumour. The mean dose rate at the tumour periphery was 48 cGy/h (27–77).

Based on previous data of LDR-IRT alone conducted by Ruifrok *et al*[18], for the combined experiments of LDR-IRT with cisplatin, the brachytherapy was given with a dose range of 20–120 Gy. As a control to the previously established dose–effect curve of the LDR-IRT, the effects of LDR-IRT alone in a dose range of 100–140 Gy were determined. During the time of irradiation animals were kept in normal housing conditions. For each dose level five or six animals were used. Sham treatment was performed by implantation of unloaded catheters during 7 days.

Drug administration

Cisplatin was dissolved and diluted in sterile water to give a solution compatible with a concentration of 3 mg/kg cisplatin body weight in a volume of 1 ml. A bolus injection of this solution was administered intraperitoneally 15 min before the start of the irradiation. As a control arm, the effect of cisplatin alone, i.e. without irradiation, was tested.

Experimental endpoints

For the determination of the tumour response, growth delay and cure have been used. Growth delay was defined as the time the tumours took to regrow to their original treatment volume, and cure if the tumour disappeared completely and no regrowth occurred during an observation period of 90 days. Tumour size was estimated by measuring three perpendicular diameters (volume $=\pi/6.d_1.d_2.d_3$), using calipers, twice weekly during the follow-up period. Weight loss was monitored as a parameter of the general condition of the animals, who were killed for ethical reasons when the tumour reached a volume of about 6 cm^3.

RESULTS

All the animals treated tolerated both the LDR-IRT alone as well as the combination of LDR-IRT with the bolus injection of cisplatin. Immediately after the irradiation, the mean weight loss was 16% (range 11–21%) and 18% (range 5–24%) of the original body weight for LDR-IRT alone and LDR-IRT in combination with cisplatin, respectively. All animals recovered and regained their normal weight after approximately 10 days.

The effects of LDR-IRT alone and LDR-IRT in combination with a bolus injection of 3 mg/kg cisplatin on tumour volume are shown in Fig. 1. In Fig. 1, the mean volume of the tumours relative to the volume at the start of the treatment at day 0 are plotted as a function of time. For reasons of clarity only IRT doses of 60, 100 and 120 Gy are shown. As can be seen, the response of the tumour to LDR-IRT alone and LDR-IRT in combination with cisplatin increased with increasing dose. The administration of an intraperitoneal bolus injection of cisplatin seemed to have had no effect on tumour response at the radiation dose levels of 100 and 120 Gy; if anything, the effects of the combined modality on tumour volume was even less as compared with the effect of 100 and 120 Gy alone. At almost all dose levels the irradiated tumours in both experiments regrew at the same growth rate as untreated tumours. Sham treatment of the tumours and the cisplatin bolus injection alone resulted in a minimal disturbance of the growth rate of the tumour during the first week, but thereafter, the tumours continued to grow with the same volume doubling time.

Figure 2 shows the time taken by the tumours to regrow to their original treatment volume (growth delay, GD_1) as a function of radiation dose. For each dose level the mean GD_1 was calculated. At dose levels of 100 and 120 Gy combined with the bolus injection of cisplatin the GD_1 was less, as opposed to GD_1 of LDR-IRT alone.

Although we had expected, from the earlier experiments with LDR-IRT alone in the R1 rhabdomyosarcoma [18], to obtain cures starting at a dose level of IRT of 60 Gy and a 100% cure probability at a dose level of 120 Gy, we found only one cure in the group of five animals irradiated to a dose of 100 Gy and two

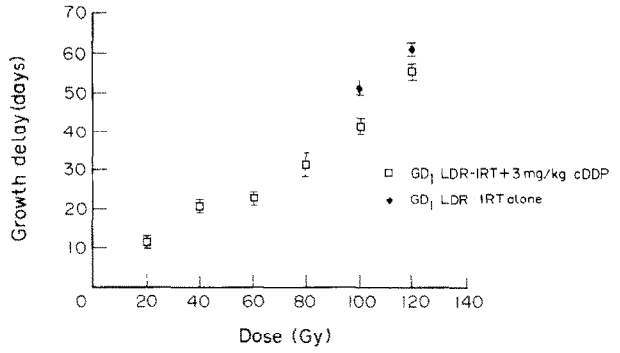

Fig. 2. Time of the tumours to regrow to their original tumour volume (growth delay=GD_t) after a range of doses of low dose rate interstitial radiotherapy alone (LDR-IRT) or combined with an intraperitoneal bolus injection of 3 mg/kg cisplatin. Error bars: S.E.M.

at a dose level of 120 Gy. Our recent experiments with LDR-IRT alone indicated that the dose–response relationship for the R1 rhabdomyosarcoma apparently had changed. A complete dose–response curve for tumour control can be given only for the LDR-IRT alone, as derived from a logit analysis. In Fig. 3, tumour control probability is plotted as a function of the radiation dose. The first cure is seen after 100 Gy, whereas for 50% tumour cure (TCD_{50}), a dose of 114 Gy (91–125 Gy, 95% confidence limit) is needed. This is in contrast with the TCD_{50} of 95 Gy, obtained in the earlier experiments for LDR-IRT alone in this tumour model. For reasons of comparison the latter is also plotted in Fig. 3, together with the cures obtained with the combined modality experiments of LDR-IRT and intraperitoneal bolus injectin of cisplatin.

Another unexpected finding was the high incidence of metastases. All animals participating in this study were checked for the incidence of metastases in lung, liver, adrenals and lymph nodes. 15 (including two control animals which had received only cisplatin) out of a total of 45 (33%) animals in the combined modality experiment of LDR-IRT with cisplatin developed metastases in the lung and/or lymph nodes of the left axilla. There was no correlation between the occurrence of metastases and time after start of treatment, or with the dose of IRT.

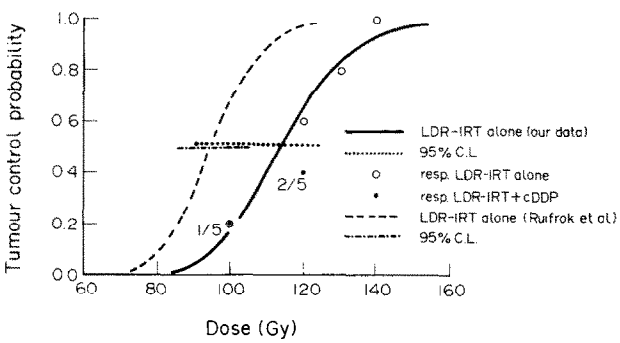

Fig. 3. Tumour control probability after low dose rate interstitial radiotherapy (LRD-IRT) alone as determined in our experiments compared with the previously published results obtained by Ruifrok *et al.* with the TCD_{50} and the 95% confidence limit shown by (....) and (-·-·-) respectively.
Number of cures/number of animals in the dose group of animals treated with low dose rate interstitial radiotherapy alone (○ = LDR-IRT alone) and at doses of 100 and 120 Gy IRT in combination with an intraperitoneal bolus injection of 3 mg/kg body weight cisplatin (● = LDR-IRT + cDDP).

DISCUSSION

We appeared to have cured one out of five animals with 100 Gy LDR-IRT combined with 3 mg/kg cisplatin and two out of five with 120 Gy LDR-IRT with 3 mg/kg cisplatin. This was in contrast to our previously reported data on using LDR-IRT alone in the same animal tumour model; i.e. of the series of animals treated by Ruifrok *et al.* [18], five out of six and six out of six cures for 100 and 120 Gy, respectively, were observed (TCD_{50}=95 Gy). We assumed that the reduced effectiveness of the combined treatment could not be attributed to the addition of cisplatin, but probably was due to a significant change in tumour characteristics. Moreover, as an unexpectedly high incidence of lung and/or axillay lymph node metastases was found, we concluded that the tumour must have dedifferentiated. It was hypothesised that the (new) tumour grew with a faster proliferation rate and obtained a higher potency to metastasise. Therefore, we again determined the dose–effect relationship of LDR-IRT alone at high dose levels. When we now compare the single modality (LRD-IRT) data regarding tumour control and growth delay at a dose level of 100 and 120 Gy with the data of the combined experiments at the same dose levels, only a small difference in favour of the LDR-IRT alone was found. This led us to conclude that in our experiments we could not establish any modifying effect to the LDR-IRT by the addition of an intraperitoneal bolus injection of cisplatin 15 min before the start of the IRT at a dose of 3 mg/kg.

There are several possibilities to explain why the combination of LDR-IRT with cisplatin in our tumour model failed to enhance the radiation effect of LDR-IRT alone. One of the explanations could be that there is no or only minimal repair of sublethal damage in the R1-rhabdomyosarcoma. This assumption could be supported by previous *in vivo* experiments performed with this animal tumour model. Kal [19] found no difference in biological effect with reduced dose rates of 75, 110 and 150 cGy/h in the R1-rhabdomyosarcoma and Vogler and Beck-Bornhold [20] observed that with increasing number of fractions, the response of the R1H-rhabdomyosarcoma was essentially independent of the number of fractions used. However, other radiobiological processes taking place during a protracted course of low dose rate irradiation, such as reoxygenation of hypoxic cells of the tumour and redistribution of the cells into a more radiosensitive phase of the cell cycle [19,21–23] could balance out the negative effect of repair on tumour control and preclude a direct comparison of these *in vivo* data. Another explanation might be that a potential interaction between the intraperitoneal bolus injection of cisplatin and LDR-IRT is not based on inhibition of sublethal damage repair. Previous results reported in literature combining LDR-IRT at different dose rates with an intraperitoneal bolus injection of cisplatin could sustain this [12,24,25]. Furthermore, we do not know in which way the dose, time sequence and mode of administration of cisplatin could influence the potential interaction with LDR-IRT in our particular tumour model. Although in other animal tumour models, the highest dose enhancement factors combining cisplatin with high dose rate irradiation have been obtained with the highest concentrations of cisplatin, administered immediately before irradiation [10,11], we had arrived at the dose of 3 mg/kg because a pilot study in our laboratory had demonstrated that a combination of 6 mg/kg cisplatin and LDR-IRT was too toxic for the animals. Finally, factors such as time sequence of drug administration, rapid plasma clearance of free platinum after a bolus injection and the low drug uptake in the tumour may all ultimately influence the concentration of the drug in the tumour,

in particular in the case of protracted courses of low dose rate irradiation. In that case, the drug may not even have the opportunity to have a significant effect on the repair of sublethal damage [12,26]. From this point of view, it would be interesting to test the combination of LDR-IRT with other administration routes of cisplatin, such as a continous infusion of the drug during the irradiation time [12,27] or intratumoral injections of cisplatin associated with a slow-release collagen-based matrix [28,29]. However, the 'drift' in our tumour model keep us from going on with this kind of combined experiments.

In conclusion, our experiments showed no therapeutic gain from combining LDR-IRT with an intraperitoneal bolus injection of 3 mg/kg body weight cisplatin, suggesting the absence of interaction between the two treatment modalities in our tumour model. However, it cannot be excluded that with other routes of administration of cisplatin, such as intratumoral injections associated with a slow-release collagen-based matrix or a continous infusion of cisplatin using Alzet osmotic minipumps for instance, the radiation response could indeed be enhanced.

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