Published in final edited form as:
Int J Radiat Biol. 2011 November ; 87(11): 1126–1134. doi:10.3109/09553002.2011.605418.

Vascular effects of plinabulin (NPI-2358) and the influence on tumour response when given alone or combined with radiation

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Abstract

Purpose—This study investigated the anti-tumour effects of the novel vascular disrupting agent plinabulin (NPI-2358) when given alone or combined with radiation.

Materials and Methods—Foot implanted C3H mammary carcinomas or leg implanted KHT sarcomas were used, with plinabulin (Nereus Pharmaceuticals, San Diego, USA) injected intraperitoneally. Dynamic contrast-enhanced magnetic resonance imaging measurements were made with gadolinium-DTPA on a 7-tesla magnet. Treatment response was assessed using either a regrowth delay (C3H mammary carcinoma) or clonogenic survival (KHT sarcoma) assay, or histological estimates of necrosis for both models.

Results—Plinabulin (7.5 mg/kg) significantly reduced both IAUC and K^{trans} within 1-hour after drug injection, reaching a nadir at 3-hours, but returning to normal within 24-hours. A dose-dependent decrease in IAUC and K^{trans}, was seen at 3-hours. No significant anti-tumour effects were seen in the C3H mammary carcinoma until doses of 12.5 mg/kg and above were achieved, but started at 1.5 mg/kg in the KHT sarcoma. Irradiating tumours 1-hour after injecting plinabulin enhanced response in both models.

Conclusions—Plinabulin induced a time and dose dependent decrease in tumour perfusion. The KHT sarcoma was more sensitive than the C3H mammary carcinoma to the anti-tumour effects of plinabulin, while radiation response was enhanced in both models.

Keywords

Plinabulin (NPI-2358); Vascular targeting; Radiation; C3H mammary carcinoma; KHT sarcoma; Magnetic Resonance Imaging

Introduction

An essential requirement for the growth and development of solid tumours is the formation of a functional vascular supply from the host normal vasculature by the process of angiogenesis (Brem et al. 1976, Folkman 1986). The significance of this tumour neo-

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vasculature makes it a potential therapeutic target and two vascular targeting approaches have been developed, one that tries to inhibit the angiogenesis process and a second that damages the tumour vessels that have been established (Siemann et al. 2005). With respect to the latter approach, a number of vascular disrupting agents (VDAs) have now been developed and many are currently in clinical evaluation (Patterson and Rustin 2007). The lead compounds in this group are agents that target the colchicine binding site of the β-tubulin monomer, and thus destabilize tubulin of the proliferating endothelial cells in the tumour as a result of a rapid depolymerization of the microtubules (Tozer et al. 2005). This effect causes subsequent rounding-up of the vascular endothelial cells, loss of tumour vascular integrity and haemorrhagic necrosis of the tumour cells (Tozer et al. 2005).

A new VDA of this type is plinabulin (NPI-2358), a synthetic analogue of the diketipiperazine phenylahistin (Nicholson et al. 2006). Phenylahistin, is a natural product isolated from *Aspergillus sp.*, which has been shown to bind to the colchicine-binding site of tubulin (Nicholson et al. 2006). Plinabulin has potent in-vitro activity against various human tumour cell lines (Nicholson et al. 2006). When tested in proliferating human umbilical vein endothelial cells (HUVECs), it was found to reduce microtubule content within 30 min at concentrations as low as 10 nmol/l, dose dependently increase HUVEC monolayer permeability, and when compared with other tubulin-depolymerizing agents with known vascular-disrupting activity (i.e., colchicines, vincristine and combretastatin) it was found to have superior or equivalent activity against HUVECs (Nicholson et al. 2006). In phase I studies plinabulin was found to be well tolerated in patients with solid tumours or lymphomas and using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) it was found to significantly reduce tumour blood flow (Mita et al. 2010a). Plinabulin is currently in phase II clinical trials in patients with non-small cell lung cancer (NSCLC) in combination with docetaxel (Mita et al. 2010b), but there are no published in vivo preclinical data for this drug.

The aim of this study was to investigate the potential in vivo activity of plinabulin by determining both the drug's ability to induce anti-vascular effects using DCE-MRI, a technique which has been suggested suitable for such estimates (Leach et al. 2003) and its anti-tumour activity by assessing tumour regrowth delay, clonogenic cell survival and the induction of necrosis. This was done in two tumour models (C3H mammary carcinoma and KHT sarcoma) which have been shown to respond differently to VDAs in terms of effects on blood perfusion, necrosis development, and anti-tumour activity (Murata et al. 2001a, Murata et al. 2001b, Nielsen et al. 2010). Since it is generally well recognized that the clinical application of vascular targeting agents will not be as a single modality, but rather in combination with conventional cytotoxic cancer therapies (Siemann et al. 2000, Horsman and Siemann 2006), the ability of plinabulin to enhance the anti-tumour activity when combined with radiation was also investigated. Again, studies were performed using both the C3H mammary carcinoma and KHT sarcoma because the enhancement of radiation response by VDAs has also been shown to be different in these two tumour models (Murata et al. 2001a, Murata et al. 2001b).

Materials and Methods

Animal and tumour models

This pre-clinical study was performed using either a C3H mammary carcinoma or the KHT sarcoma, the derivation and maintenance of which have been described in detail previously (Overgaard 1980, Kallman et al. 1967). C3H mammary carcinomas do not grow in culture, thus experimental tumours were produced following sterile dissection of large flank tumours. Macroscopically viable tumour tissue was minced with scissors and 5–10 ul of this material injected into the right rear foot of 10–14-week-old female CDF1 mice. Experiments

were performed when tumors had reached approximately 200 mm^3 in size, which typically occurred 3 weeks after inoculation. Tumor volume was calculated from the formula D1 × D2 × D3 × π/6, where the D values represent the three orthogonal diameters. KHT sarcoma cells were passaged in vivo in 8-weeks-old female C3H/He mice. Experimental tumours were produced by injecting 2×10^5 cells, dissociated from such tumours, intramuscularly into the hind limb of recipient mice. Experiments were performed about two weeks later when tumours had reached about 500–700 mg in weight. All experiments were performed using non-anesthetized animals and were conducted under institutionally and nationally approved guidelines for animal welfare.

Drug preparation

Plinabulin was supplied by Nereus Pharmaceuticals (San Diego, California, USA). It was freshly prepared before each experiment in a polyethylene glycol/solutol solution and diluted to the required concentration with 5% dextrose. Stock drug solutions were kept cold and protected from light. Plinabulin was injected intraperitoneally (i.p.) in a volume of 0.02 ml/g mouse body weight in CDF1 mice and 0.01 ml/g body weight for C3H/Hej mice.

Magnetic resonance imaging

A 7-Tesla spectroscopy/imaging system (Varian Medical Systems, Palo Alto, CA) was used for dynamic contrast enhanced- magnetic resonance imaging (DCE-MRI) as previously described (Nielsen et al. 2008). Basically, non-anesthetized C3H mammary carcinoma bearing mice were restrained in specially constructed Lucite jigs and their tumour bearing legs exposed and loosely attached to the jig with tape, without impairing the blood supply to the foot. A cannula, connected by way of a 0.38-mm inner diameter line, 80 cm in length, to a syringe primed with contrast agent solution, was intravenously (i.v.) inserted into the mouse tail vein and held in place with tape. An i.p. line similar to the i.v. line was connected to a syringe primed with plinabulin solution. The jig was positioned in a cradle containing an 11-mm surface coil and tuning box, the tumour was located under the coil, and a warm water tube was wrapped around the jig.

A single slice along the foot through the tumor center was chosen for imaging. The imaging protocol included an inversion recovery sequence for T_1 mapping (field of view, 25 × 25 mm; slice thickness, 2 mm; matrix size, 128 × 64, reconstructed to 128 × 128; repetition time, 2,430 ms; inversion times, 100, 400, 800, 1,600, and 2,400 ms; and echo time, 13 ms), followed by dynamic image acquisition using a fast spoiled gradient echo sequence (field of view, 25 × 25 mm; slice thickness, 2 mm; matrix size, 128 × 100, reconstructed to 128 × 128; flip angle, 70°; repetition time, 60 ms; and echo time, 3 ms). This gave a time resolution of 6 s for the dynamic images. During the initial 4 s of the sixth image acquisition, gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) (Magnevist, Schering, Berlin, Germany) was administered i.v. at a dose of 0.1 mmol/kg and a concentration of 0.02 mmol/ml. One hour after Gd-DTPA administration, plinabulin was i.p. injected. The mice were kept in place and repeated images were performed on the same animals either at 1, 3, and 6 hours (time course study) or only at 3 hours (dose response study) after giving plinabulin. For the time course study, animals were returned to their cages after the 6 hour measurement, but repositioned in the magnet on the following day, as described above, so that a 24 hour measurement could be obtained.

For each tumor, a region of interest (ROI) containing the whole tumor was drawn manually for the data analysis. T_1 maps were calculated from the pre- and post-plinabulin inversion recovery images. We assumed a linear relationship between the Gd-DTPA concentration and ΔR_1 ($R_1 = 1/T_1$), with a relaxivity $r_1 = 2.92\ mM^{-1}s^{-1}$ [8] (Pedersen et al. 2000). It was further assumed that Gd-DTPA affected the relaxation rate of all tissue water molecules (so-

called fast water exchange: water moves freely over vascular and cellular boundaries over the time-scale of the image acquisition). The signal intensity in the dynamic images was then converted to the Gd-DTPA concentration using the signal equation for the dynamic images to produce Gd-DTPA concentration-time curves for each voxel in the ROI.

The voxel concentration-time curves were used to calculate maps of the vascular parameters. The semiquantitative parameter initial area under the curve (IAUC) was calculated by trapezoidal integration of the Gd-DTPA concentration during the first 90 s after administration. Tumor voxels with low initial contrast agent uptake could not be used for model analysis and were assumed to be necrotic. Therefore, voxels with IAUC values of<0.0005 Ms were excluded from IAUC ROIs, and the following quantitative analysis for supporting consistency between IAUC and model ROIs.

The standard DCE-MRI model, including a vascular term (three-parameter model), was fitted to the curves for quantitative estimation of the transfer constant K^{trans}, the rate constant k_{ep}, the extravascular extracellular space, $v_e = K^{trans}/k_{ep}$, and the plasma volume fraction, v_p (Tofts et al. 1999):

$$C_t(t)=K^{trans}\int_0^t C_p(T)e^{-kep(t-T)}dT+v_pC_p(t) \quad (1)$$

Where $C_t(t)$ was the tissue concentration of Gd-DTPA and $C_p(t)$ was the plasma concentration of Gd-DTPA. The same model without the vascular term (two-parameter model) was also applied. The tracer plasma concentration $C_p(t) = D(a_1e^{-m1t} + a_2e^{-m2t})$ was based on measurements by Furman-Haran and colleagues (Furman-Haran et al. 1997) and adapted in this experiment. The published $m_1 = 0.00717\ s^{-1}$, $m_2 = 0.00095\ s^{-1}$, and $a_1/a_2 = 1.3437$were used, along with the current dose D = 0.0001 mol/kg and mass per plasma $a_1 + a_2 = 30.17$ kg/L, assuming a blood volume of 65 mL/kg and a previously measured haematocrit of 0.49 (Horsman et al. 2003).

Fitting was performed using nonlinear least squares minimization in MATLAB, version 7.4 (The MathWorks, Natick, MA). Both models were fitted for each voxel in the IAUC ROI in which the voxels with low contrast uptake were excluded. The criteria for an acceptable fit were positive parameter values and a mean fit point distance to the measured points ≥ 0.5 M. Voxels not satisfying these criteria were excluded from the model ROIs. In voxels in which a two-parameter fit was superior to a three-parameter fit, this was most likely a result of low plasma volume. Therefore, the values of the two-parameter model were adopted into the three-parameter model with v_p set to 0. When voxels showed an acceptable fit before treatment only, this was most likely a result of complete vascular shutdown after treatment, making model parameter estimation impossible. However, exclusion of these voxels would underestimate the drug effect. Therefore, such voxels were included with the post-treatment K^{trans}, K_{ep} and v_p set to 0. However, the model parameter $v_e = K^{trans}/k_{ep}$ was then indeterminate after treatment in these voxels. As a consequence, the biologic changes in the extra-vascular extracellular space could not be estimated. Therefore, we compared the pre- and post-treatment v_e by a ROI, in which only voxels having satisfying model fits at both points were included.

Anti-tumour activity of plinabulin

The ability of plinabulin to induce anti-tumour effects was assessed using two different assays depending on the tumour model. With the C3H mammary carcinoma a tumour regrowth delay assay was used (Horsman and Murata 2002). This involved measuring tumor volume 5 times each week after treatment, as described earlier, and calculating the tumor growth time (TGT; time in days to reach 3 times the treatment volume). For the KHT

sarcoma response was determined using an in vivo/in vitro clonogenic cell survival assay as previously described (Siemann 1995). Briefly, whole tumours were excised 24 hours after treatment, and single-cell suspensions were prepared from the tumors using a combined mechanical and enzymatic dissociation procedure. The cells were counted on a haemacytometer and various dilutions were prepared. These cells were then mixed with 10^4 lethally irradiated tumour cells in 0.2% agar containing alpha-minimum essential medium supplemented with 10% fetal calf serum and plated onto 24-well multiwall plates. These plates were harvested two weeks later, and the resulting colonies counted with the aid of a dissecting microscope. Tumour surviving fractions were determined by multiplying the calculated fraction of surviving cells by the ratio of the numbers of cells recovered from treated and untreated tumours. The plating efficiency of untreated tumour cells was approximately 20%.

Necrotic fraction estimates

The procedure used has been described (Horsman et al. 2000). Essentially, mice were killed by cervical dislocation 24 h after injecting plinabulin and their tumors excised. They were then fixed in formalin and haematoxylin and eosin-stained sections made. A randomly selected section was cut, and 2–4 additional, equally spaced sections were produced and examined under a projecting microscope. Each section was systematically scanned and projected on a grid with equidistant-spaced points. For each field of vision, the total number of points hitting the tumor (nT) and necrosis (nN) were recorded and the necrotic fraction of the tumor was defined by Σ nN / Σ nT.

Tumour irradiation and response

The C3H mammary carcinomas were irradiated using a conventional therapeutic 230 kV X-ray machine at a dose rate of 2.3 Gy/min as previously described (Horsman et al. 1989). All irradiations were given locally to the tumors. To achieve this, non-anesthetized mice were restrained in Lucite jigs, with their tumor-bearing legs exposed and loosely attached to the jig with tape, as described for the DCE-MRI studies. To secure homogeneity of the radiation dose, the tumours were immersed in a water bath at 25°C with about 5 cm of water between the X-ray source and the tumour. Tumours only were irradiated, the remainder of the mouse being shielded by 1 cm of lead. With this set-up three mice could be simultaneously irradiated. Response to treatment was assessed using a regrowth delay assay. For the KHT tumours, irradiations were performed as previously described (Siemann et al. 1977) using a ^{137}Cs source operating at a dose rate of 1.5 Gy/min. Each mouse was confined to a plastic jig with its tumour-bearing leg extended through an opening in the side to allow the tumor to be locally irradiated. Five tumour-bearing animals could be irradiated simultaneously. Treatment response was determined using the in vivo/in vitro clonogenic cell survival assay.

Statistical analysis

All results are shown as means (±1 S.E.). Statistical analyses of the data were performed using either a Student’s t-test after testing for variance homogeneity using an F-test, or using Mann-Whitney signed-rank test. For either test a significance level of $p < 0.05$ was selected.

Results

Figure 1 illustrates the change in IAUC in the C3H mammary carcinoma model as a function of time after treatment in either control treated animals or mice given a single i.p. injection of 7.5 mg/kg plinabulin. The mean (±1 SE) IAUC value for untreated mice was 0.012 (0.011–0.014). IAUC showed a non-significant decrease during the first 6 hours of measurement in the control animals that were injected with the drug solvent, probably related to the non-anaesthetised, but restrained, animals simply remaining in the magnet for

this entire time period. There was a slight, but non-significant rise at 24 hours, which is probably the result of removing the animals from the magnet after 6 hours and then re-positioning them for the 24 hour measurement. Following injection with plinabulin there was a significant decrease in IAUC with values reaching 62% (57–67) and 56% (45–69) of the control values at 1 and 3 hours, respectively. However, at 6 and 24 hours after drug injection there was no significant difference in IAUC between controls and drug treated animals, with the respective values after plinabulin treatment being at 80% (73–87) and 78% (66–90) of controls. Similar changes were seen with K^{trans} (excluding plasma volume), although at 6 hours after injecting plinabulin there was still a significant difference compared to controls, and in v_p (including plasma volume), but not with any of the other parameters (Table 1).

The plinabulin dose-response effects obtained when measurements were made at the optimal time of 3 hours after drug injection are also shown in Figure 1 and Table 2. IAUC decreased in a dose dependent fashion with respective IAUC values of 84 % (75–90), 81 % (64–96), 56 % (45–69), and 44 % (36–52) of the control values for 2.5, 5.0, 7.5, and 10.0 mg/kg. However, statistical significance was only seen at the 7.5 mg/kg dose and above, and at doses greater than 10 mg/kg no additional decrease was observed. As with the time-course study similar changes were found with K^{trans} (excluding plasma volume) and v_p (including plasma volume), but not the other DCE-MRI parameters (Table 2).

The dose dependent effect of plinabulin on tumour regrowth and necrosis induction in the C3H mammary carcinoma is shown in Figure 2. Untreated controls had a mean (± 1 SE) TGT3 of 4.3 days (4.1–4.5). This was unaffected by drug doses at 10 mg/kg and below, but was increased to 5.1 days (4.9–5.2) and 5.3 days (5.1–5.5) at 12.5 and 15.0 mg/kg, respectively, although it was only significant at the highest dose. Measurement of tumour necrosis in this model showed similar results with the control level of 21% (16–25) unchanged at 10 mg/kg and below, but significantly increased to 34% (31–38) and 39% (34–45) for 12.5 and 15.0 mg/kg doses, respectively. Figure 2 also shows the effect of combining plinabulin with radiation on tumour regrowth delay. In these experiments the drug was given as a single i.p. injection 1 hour after irradiation. As was the case for the dose response for the drug alone the TGT3 of 11.2 days (10.8–11.6) for radiation alone was unchanged until doses above 10 mg/kg were given, the respective values being 13.2 (12.0–14.5) and 12.4 (12.1–12.7) at 12.5 and 15. 0 mg/kg, and both these increases were significantly different from radiation alone.

Similar drug-dose response studies were performed using the KHT sarcoma and the results presented in Figure 3. In this tumour model cell killing by plinabulin increased with increasing drug dose, with an effect seen even using the lowest drug tested of 1.5 mg/kg, and still increasing at the highest drug dose used (10 mg/kg). A similar dose-response trend was seen in the estimates of necrotic fraction in this tumour, with the percent necrosis increasing from a value of 14% (11–17) in control tumours to 91% (86–96) in tumours treated with a plinabulin dose of 10 mg/kg. Also included in Figure 3 is a radiation dose response curve in which KHT tumours were either irradiated alone or 1 hour before a single injection of 2.5 mg/kg plinabulin. The drug alone reduced tumour cell survival by almost 2 log-units and when combined with radiation there appeared to be a simple parallel downward shift in the radiation dose response curve.

Discussion

DCE-MRI is an established technique suitable for identifying vascular changes induced by both angiogenesis inhibitors and VDAs (Leach et al. 2003). Using gadolinium-DTPA as the contrast agent we showed time and dose-dependent vascular mediated changes after

treatment with plinabulin in the C3H mammary carcinoma model. Perfusion decreased rapidly after drug injection with significant decreases seen within 1-hour after drug injection, and reaching a nadir by 3-hours. At longer time intervals perfusion recovered, although exactly when this occurred depended on the DCE-MRI parameter used, but was complete within 24 hours in all cases. Similar rapid changes in perfusion have been seen with other tubulin depolymerizing agents in this tumour model. These include the lead drug in clinical trials combretastatin A-4 phosphate (Maxwell et al. 1998, Murata et al. 2001c, Bentzen et al. 2005, Breidahl et al. 2006, Ley et al. 2007, Nielsen et al. 2008), its analogue OXi4503 (Horsman unpublished observations), and ZD6126 (Horsman and Murata 2003, Breidahl et al. 2006). The techniques used to show these perfusion changes include RbCl uptake (Murata et al. 2001c, Horsman and Murata 2003), laser Döppler flowmetry (Ley et al. 2007), magnetic resonance spectroscopy/imaging (Maxwell et al. 1998, Breidahl et al. 2006), and DCE-MRI (Bentzen et al. 2005, Nielsen et al. 2008, Horsman unpublished observations). Using the DCE-MRI technique in human patients, plinabulin has been shown to significantly decrease tumour blood perfusion in the dose range of 13.5–30 mg/m^2 (Mita et al. 2010a), similar to that used in the present studies (7.5 mg/kg is equivalent to 21.5 mg/m^2 in the mouse).

For the DCE-MRI dose-response study measurements were made 3-hours after drug injection, and showed increasing effects with increasing drug dose, reaching significance at 7.5 mg/kg and being maximal at 10 mg/kg and above. The relationship between plinabulin dose and the development of necrosis and growth inhibition in this C3H mammary carcinoma model were also investigated. Using either endpoint, no significant changes were observed until the highest doses tested (12.5 and 15 mg/kg) were achieved. Clearly, there is a slight differentiation between the DCE-MRI measurements and the anti-tumour effects induced by plinabulin. The DCE-MRI measurements were performed at the time of the maximal decrease in the gadolinium-DTPA tracer, but a time course estimate was only performed with one drug dose (7.5 mg/kg) and it is possible that at the higher doses the vascular effects of plinabulin are of longer duration. It is possible that the more relevant endpoint for non-invasively estimating drug activity is the length of time by which perfusion is reduced once the maximal decrease has occurred rather than simply measuring the maximal decrease per se. In retrospect, monitoring the DCE-MRI changes at both 3 and 24 hours would have been preferable.

The anti-tumour activity of plinabulin was apparent at much lower doses (effective at doses of 1.5 mg/kg and above) in the KHT sarcoma using the endpoints of tumour necrosis induction and changes in tumour cell survival. This difference in tumour model sensitivity to VDAs has been previously reported (Murata et al. 2001a, Murata et al. 2001b, Nielsen et al. 2010). As the drug dose increased so did the anti-tumour effects and as with the C3H mammary carcinoma the change in necrosis induction and the anti-tumour effects generally correlate.

Despite seeing significant anti-tumour effects with plinabulin in both the C3H mammary carcinoma and KHT sarcoma its use as monotherapy is unlikely. The consensus opinion is that for any vascular targeting drug, whether its mode of action is to inhibit angiogenesis or initiate vascular disruption, the potential clinical application will be in combination with more conventional anti-cancer therapies (Siemann et al. 2000, Horsman and Siemann 2006). Indeed, phase II clinical trials with plinabulin are now being conducted in NSCLC in combination with docetaxel (Mita et al. 2010b). Pre-clinical studies have demonstrated that VDA treatment produces effects that even when severe are typically restricted to the central part of the tumour, leaving a rim of viable tumour cells at the periphery (Dark et al. 1997, Lash et al. 1998, Li et al. 1998, Grosios et al. 1999, Siemann and Rojiani 2002). This occurs presumably because the tumour rim receives its nutritional support from the nearby normal

tissue blood vessels which are generally unaffected by treatment with VDAs (Chaplin et al. 1999, Siemann 2002). Cells in the tumour periphery are thus more likely be better oxygenated and as such more sensitive to radiation therapy, making the combination of VDAs with radiation a logical therapeutic application. Numerous pre-clinical studies have now demonstrated the benefit of combining VDAs with radiation (Siemann et al. 2000, Horsman and Siemann 2006). Our own measurements of tumour necrosis suggested more centrally located effects by plinabulin (data not shown), and consistent with that we found a larger benefit when radiation and plinabulin were combined. For both the C3H mammary carcinoma and the KHT sarcoma this enhancement appeared to be no greater than additive of the effect found with the drug and radiation alone, which is to be expected given that each treatment attacks separate anatomical regions of the tumour.

In our study, plinabulin was only administered at a single time point after irradiating the tumours. Other studies with VDAs have investigated the importance of sequence and timing between VDA and radiation treatments (Wilson et al. 1998, Murata et al. 2001a, Murata et al. 2001b, Siemann and Rojiani 2002). These studies indicated that injecting the VDA prior to irradiating gave little or no benefit, and sometimes there was even a suggestion of a less than additive response, probably indicating the induction of hypoxia following vascular shut-down. The fact that the bioreductive drug tirapazamine could be used to further improve the efficacy of the VDA DMXAA with radiation supports such a suggestion (Wilson et al. 1998). The greatest effect of combining VDAs with radiation was always observed when the VDA was administered within a few hours after irradiating (Wilson et al. 1998, Murata et al. 2001a, Murata et al. 2001b, Siemann and Rojiani 2002), which adds support to the time interval used in the current study. While such a schedule has sometimes resulted in a greater than additive response (Wilson et al. 1998, Murata et al. 2001a, Murata et al. 2001b, Siemann and Rojiani 2002) this was not the case in the present investigation. Nonetheless, an additive response between plinabulin and radiation would clearly be beneficial, provided there was no drug enhancement of radiation-induced normal tissue reactions. Although this was not investigated in the current study, our prior experience indicates no reason to believe that such a combination would result in increased normal tissue reactions. Pre-clinical studies in mouse models, including the same CDF1 mouse strain used for the C3H mammary carcinoma experiments, found no increase in early responding skin response when combretastatin A-4 phosphate (Murata et al. 2001a) or DMXAA (Wilson et al. 1998, Murata et al. 2001b) were combined with radiation and no effect of this combination on the more critical late reactions measured in bladder and lung (Horsman et al. 2002).

In conclusion, the current study has shown plinabulin, a novel agent acting at the tubulin binding site of docetaxel, to have activity against tumour vasculature and anti-tumour activity as a single agent. It also significantly enhanced the effect of radiation therapy. These results, therefore, lend additional support to the further clinical development of this agent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Ms. Dorthe Grand, Ms. Pia Schjerbeck, Ms. Inger Marie Horsman, Mr. Mogens Johannsen and Mr. Chris Campo for their excellent technical assistance. This work was supported by funding from Nereus Pharmaceuticals, and grants from the Danish Cancer Society, and the National Cancer Institute (Public Health Service Grant CA084408).

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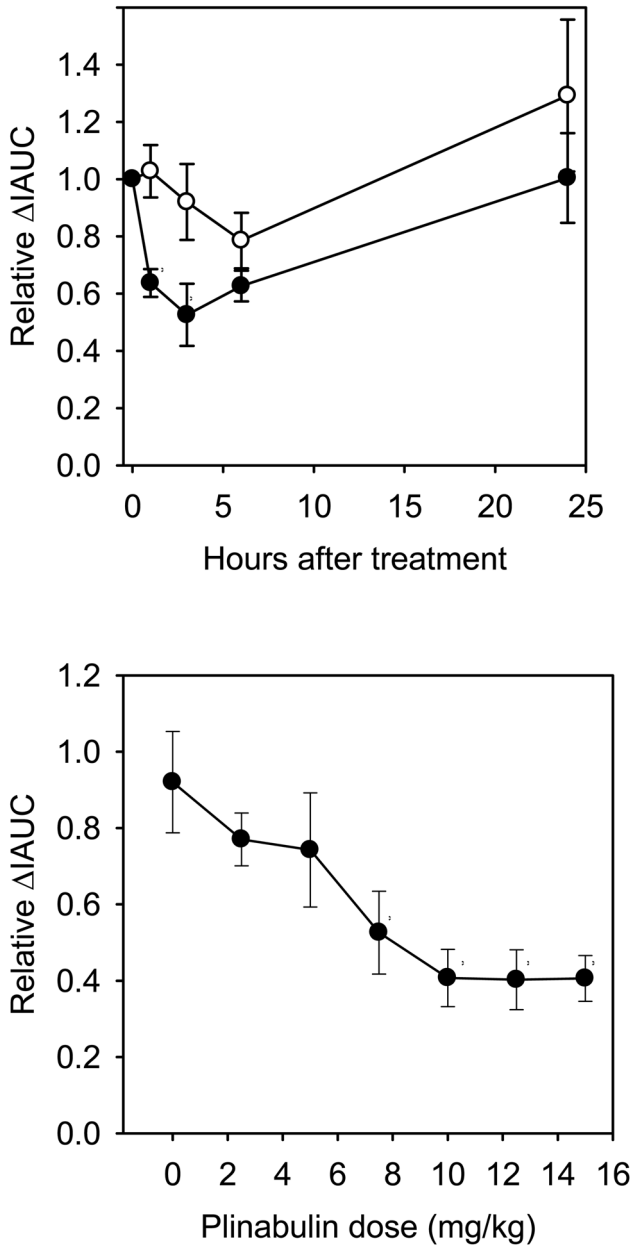

Figure 1.

Time- and dose-dependent changes in IAUC induced by plinabulin in a C3H mammary carcinoma. DCE-MRI measurements were made pre- and post-treatment with either drug solvent or plinabulin and the post-treatment change relative to the pre-treatment estimate, determined. *Top panel:* Repeated measurements were made in the same animals receiving drug solvent (○) or 7.5 mg/kg plinabulin (●). *Bottom panel:* Measurements were made 3 hours after injecting different plinabulin doses. Results show mean values (±1 S.E.) from at least 6 animals. Asterisks indicate those values that are statistically different from controls.

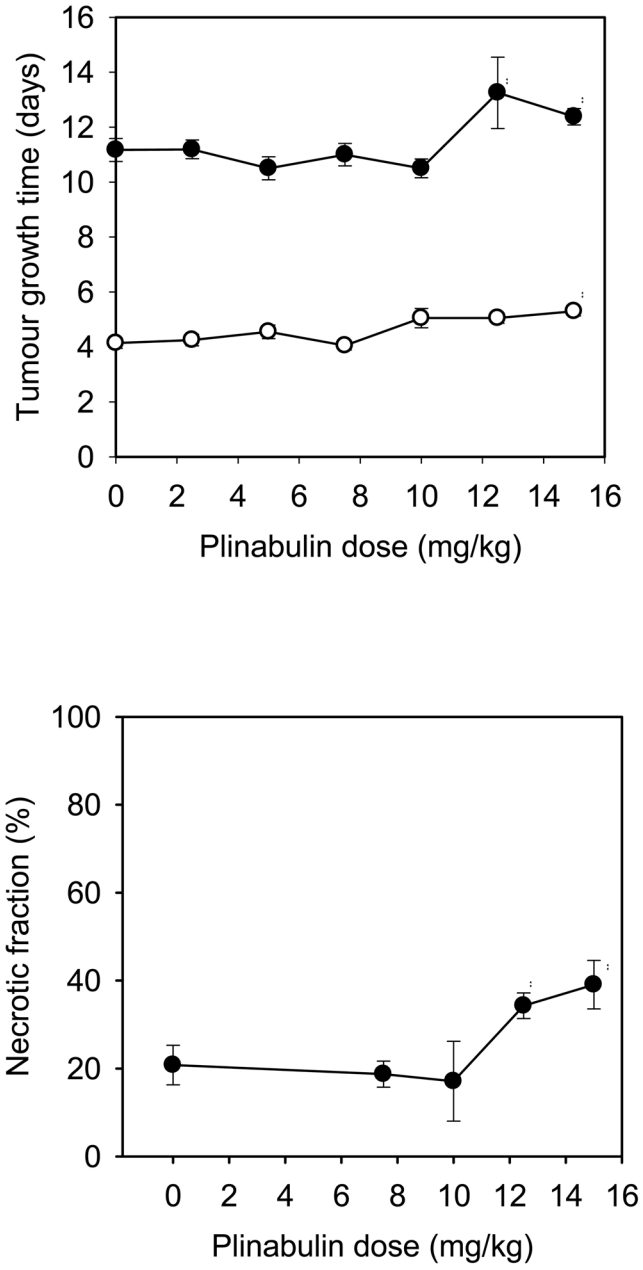

Figure 2.

The effect of plinabulin on tumour growth time and the induction of necrosis in a C3H mammary carcinoma. *Top panel:* The time for tumours to reach 3 times the treatment volume (TGT3) was determined as a function of plinabulin dose in mice injected with either drug alone (○) or when the drug was given 1 hour after local tumour irradiation with 10 Gy (●). *Bottom panel:* The degree of tumour necrosis was determined from histological sections 24 hours after injecting plinabulin. Results show mean values (± 1 S.E.) from at least 6 animals. Asterisks indicate those values that are statistically different from controls.

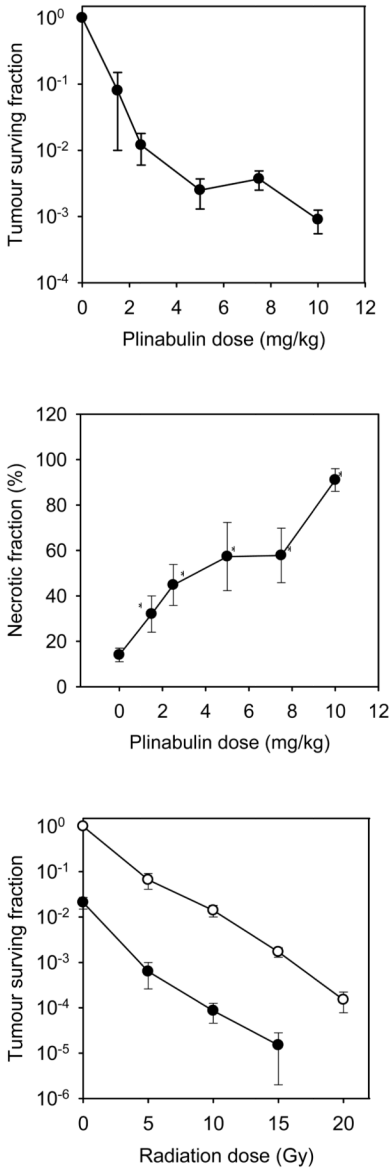

Figure 3.

The effect of plinabulin on tumour surviving fraction and the induction of necrosis in the KHT sarcoma. *Top panel:* Tumour surviving fraction was determined using the clonogenic survival assay 24 hours after injecting different doses of plinabulin. *Middle panel:* The degree of tumour necrosis was determined from histological sections 24 hours after injecting plinabulin. *Bottom panel:* Tumour surviving fraction was determined using the clonogenic survival assay 24 hours after local tumour irradiation with different radiation doses either given alone (○) or 1 hour prior to injecting the mice with 2.5 mg/kg plinabulin (●). Results show mean values (± 1 S.E.) from at least 3 animals. Asterisks indicate values statistically different from controls.

Table 1

Plinabulin induced time-dependent changes in DCE-MRI parameters relative to pre-treatment values

(A) 0 mg/kg					
	Excluding plasma volume		Including plasma volume		
Time	K^{trans}	v_e	K^{trans}	v_e	v_p
0 hour	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
1 hour	1.08 ± 0.19	1.05 ± 0.10	0.96 ± 0.07	1.02 ± 0.10	1.01 ± 0.12
3 hour	0.81 ± 0.11	1.05 ± 0.16	0.81 ± 0.07	1.10 ± 0.20	0.85 ± 0.17
6 hour	0.60 ± 0.06	1.02 ± 0.15	0.75 ± 0.06	1.12 ± 0.20	0.67 ± 0.14
24 hour	1.51 ± 0.29	1.54 ± 0.44	1.65 ± 0.28	1.58 ± 0.56	0.97 ± 0.31

(B) 7.5 mg/kg					
	Excluding plasma volume		Including plasma volume		
Time	K^{trans}	v_e	K^{trans}	v_e	v_p
0 hour	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
1 hour	0.51 ± 0.09 *	0.92 ± 0.06	0.91 ± 0.10	0.99 ± 0.08	0.44 ± 0.07 *
3 hour	0.24 ± 0.09 *	0.93 ± 0.10	0.62 ± 0.10	1.07 ± 0.11	0.33 ± 0.11 *
6 hour	0.43 ± 0.05 *	0.99 ± 0.06	0.88 ± 0.09	1.09 ± 0.08	0.39 ± 0.05
24 hour	1.12 ± 0.34	1.46 ± 0.29	1.92 ± 0.44	1.57 ± 0.32	0.63 ± 0.18

All results show means (±1 S.E.) for at least 6 animals.

* Indicates statistical differences between the values obtained with 0 mg/kg and 7.5 mg/kg plinabulin at the same time point.