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ET_B receptor agonist, IRL 1620, does not affect paclitaxel plasma pharmacokinetics in breast tumour bearing rats

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Abstract

Endothelins are potent endogenous vasoactive substances. We have found that intravenous administration of endothelin $(ET)_B$ receptor agonist, IRL 1620 (*N*-suc-[Glu^9, Ala^{11,15}]ET-1 (8–21)) to tumour bearing rats increases blood perfusion and enhances delivery of chemotherapeutic agents to the tumour tissue. This study was conducted to determine whether IRL 1620, an ET_B receptor selective agonist, alters pharmacokinetics of paclitaxel in breast tumour bearing rats. Breast tumours were induced in female Sprague-Dawley rats by *N*-methyl-n-nitrosourea (50 mg kg^{-1}, i.p). Saline (0.3 mL kg^{-1}, i.v.) or IRL 1620 (3 nmol kg^{-1}, i.v.), was administered to the tumour bearing rats via the tail vein. Paclitaxel (3 mg kg^{-1}, i.v.) was administered 15 min after saline or IRL 1620 injection. Serial plasma samples were collected up to 10 h after paclitaxel administration and analysed using an HPLC-UV assay. In a similar study [^{3}H]-paclitaxel (40 μCi, i.v.) was administered after saline or IRL 1620 injection as described above and serial plasma samples were collected until 24 h. Data was fitted to a three-compartment model and pharmacokinetic parameters were generated using WinNonlin software. The $AUC_{0-\infty}$ (9.42 ± 3.18 μg h mL^{-1}), clearance (0.69 ± 0.17 L h^{-1} kg^{-1}), volume of distribution (10.31 ± 4.54 L kg^{-1}) and half life (1.00 ± 0.32 h) of [^{3}H]-paclitaxel in tumour rats were similar in rats treated with IRL 1620 or vehicle. Tumour concentration of [^{3}H]-paclitaxel was determined in rats treated with IRL 1620 or vehicle and there was a significant increase in tumour paclitaxel concentration (308.59 ± 24.42%) in rats treated with IRL 1620 compared with vehicle. It is concluded that IRL 1620, an ET_B receptor agonist, does not alter paclitaxel pharmacokinetics and can selectively augment the delivery of paclitaxel to the tumour tissue.

Introduction

Studies have found an over-expression of endothelin (ET) receptors in breast tumour tissues and cell lines (Alanen et al 2000). ET-1 is a vasoactive substance and acts on two subtypes of receptors – ET_A receptors, which are responsible for vasoconstriction and ET_B receptors, which are responsible for vasodilatation (Inoue et al 1989; Secombe & Schaff 1994). Recently, we have shown that ET-1 produces an increase in blood perfusion to the breast tumour (Rai & Gulati 2003). This effect is attenuated by BQ788, an ET_B receptor antagonist, confirming that ET_B receptors are involved in vasodilatation induced by ET-1 in the breast tumour (Rai & Gulati 2003). An increase in perfusion to the breast tumour was found to be selective to the tumour, while other organs of the body were minimally affected. Therefore, ET-1 induced vasodilatation appears to be selective to the breast tumour. These findings can provide us with a unique tool to deliver anti-cancer drugs selectively to the tumour tissue. Further studies conducted in our laboratory have shown that IRL 1620 (*N*-suc-[Glu^9, Ala^{11,15}]ET-1 (8–21)), a highly selective ET_B receptor agonist, significantly increases tumour perfusion (Rai et al 2004). We have successfully used this vasodilating agent to selectively enhance the delivery of paclitaxel to the breast tumour (Rai et al 2004).

Paclitaxel has shown significant activity against metastatic breast cancer, refractory ovarian cancer, non-small cell lung cancer, AIDS related Kaposi's sarcoma and head and neck cancers (Rowinsky et al 1990; Holmes 1995; Huizing et al 1995; Wall et al 1995; Fitzpatrick & Wheeler 2003). We have chosen paclitaxel for its rapid transcellular transport and mechanism of action as well as its lipophilicity, which enhances

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tissue binding (Walle & Walle 1998). Previous studies from our laboratory indicate that administration of paclitaxel during the elevated perfusion results in a concomitant increase in paclitaxel concentration in the tumour (Rai et al 2004). This increase was selective for the tumour tissue, as it was not seen in any other major organ systems (Rai et al 2004).

Altering the blood flow dynamics in the body can significantly affect the pharmacokinetics of the therapeutic moiety. Paclitaxel is known to have complex pharmacokinetic properties. It is therefore important to understand the impact of IRL 1620 on the plasma pharmacokinetics of paclitaxel. Paclitaxel displays extensive tissue distribution, high plasma protein binding, variable systemic clearance and minimal renal excretion. It has a large volume of distribution ($Vdss = 50–400\,L\,m^{-2}$) (Longnecker et al 1987; Wiernik et al 1987b; Brown et al 1991; Sonnichsen & Relling 1994). This study was conducted to determine whether IRL 1620, an ET_B receptor selective agonist, alters pharmacokinetics of paclitaxel in breast tumour bearing rats.

Materials and Methods

Drugs

N-Suc-[Glu^{9}, Ala^{11,15}]ET-1 (8–21) (IRL 1620) was purchased from Sigma-Aldrich (St Louis, MO, USA). Paclitaxel ($6\,mg\,mL^{-1}$ solution; Ben Venue Laboratories Inc., Bedford, OH, USA), ketamine (Phoenix Scientific, Inc., St Joseph, MO, USA) and xylazine (Phoenix Scientific, Inc., St Joseph, MO, USA) were purchased from the UIC Pharmacy (Chicago, IL). [^{3}H]-Paclitaxel ($1\,mCi$, $6.4\,Ci\,mmol^{-1}$, specific activity) was purchased from Moravek Biochemicals (CA, USA). Urethane was purchased from Sigma Aldrich (Sigma Chemicals, St Louis, MO, USA).

Animals

Virgin female Sprague-Dawley rats (Harlan Co., Madison, WI, USA), 48 days old (120–140 g), were used for this study. Upon arrival at the Biological Research Laboratories (BRL) at UIC, all rats were housed three to a cage, in a room with controlled temperature ($23 \pm 1°C$), humidity ($50 \pm 10\%$) and artificial light (0600–1800 h). The rats had free access to food and water. The experiments were begun only after the rats were acclimatized to the environment for at least 4 days. The Animal Care Committee of UIC approved the protocols, and all rats were used according to the rules and guidelines of the American Association for Accreditation of Laboratory Animal Care (AAALAC).

Tumour induction

N-methyl-n-nitrosourea (MNU) is one of the most commonly used carcinogens to induce tumours in mammary glands (Liska et al 2000; Macejova & Brtko 2001). MNU was administered at a dose of $50\,mg\,kg^{-1}$, i.p. Rats were palpated twice weekly to monitor tumour appearance. Experiments were performed once tumours reached a minimal size of $100\,mm^3$. This tumour model is best suited as a comparative study with human solid tumours as it is also autochthonous with a generous supply of blood vessels (Liska et al 2000).

Determination of paclitaxel pharmacokinetics in normal and tumour bearing rats using HPLC-UV

Anaesthesia and surgery

Rats were anaesthetized with a single intraperitoneal injection of urethane ($1.5\,mg\,kg^{-1}$) (Sigma Chemicals, St Louis, MO, USA). The right femoral region was shaved and cleaned with surgical disinfectant and alcohol. The right femoral artery and vein were exposed and cannulated with sterile PE-50 tubing. The neck was shaved and cleaned with surgical disinfectant and alcohol. A midline incision was made in the neck region and the trachea was intubated and connected to a rodent ventilator (Model 683; Harvard Apparatus Inc., South Natick, MA). All surgery was performed under aseptic conditions. Neosporin antibiotic cream (Pfizer, Morris Plains, NJ) was applied to the wounds to prevent infection. A 45-min recovery period was allowed before drug administration.

Drug administration

Normal and tumour bearing rats were used ($n = 4$ in each group). Paclitaxel was given intravenously ($3\,mg\,kg^{-1}$) 15 min after IRL 1620 ($3\,nmol\,kg^{-1}$) or vehicle (saline, $0.3\,mL\,kg^{-1}$) administration. A 15-min time interval was selected based on tumour perfusion studies conducted previously, where IRL 1620 ($3\,nmol\,kg^{-1}$) induced a maximal increase in tumour perfusion at 15 min post infusion (Rai et al 2004). Time-lag studies revealed that the optimal time for paclitaxel administration was 15 min after IRL 1620 to achieve the highest concentration in the tumour (Rai et al 2004). Blood was collected before IRL 1620 administration to provide baseline values. Blood (0.5 mL) was drawn from the rats in heparinized syringes at baseline, 5 min, 30 min, 2 h, 6 h and 10 h after paclitaxel administration. The samples were centrifuged and plasma was harvested and stored at $-80°C$ until analysis.

Paclitaxel analysis

Plasma samples were analysed for paclitaxel using an HPLC system. Briefly, plasma was thawed and mixed with $50\,\mu L$ of the internal standard N-cyclohexyl benzamide (3 mM, lower standard curve and 30 mM, higher standard curve) and 3 mL of ethyl ether (Fisher Scientific, Chicago, IL) in a 13×100 glass culture tube. The mixture was shaken using a reciprocal shaker for 5 min and then centrifuged for 5 min at $3000\,rev\,min^{-1}$ at 4°C. The resulting supernatant was transferred to a 13×100 borosilicate glass culture tube and evaporated under a stream of nitrogen in a heated water bath (37°C). The residue was reconstituted with $200\,\mu L$ of mobile phase A (50% de-ionized water, 50% acetonitrile). A 100-μL (lower standard curve

and samples collected after intravenous administration) volume of the reconstituted material was injected into a 4-mm NovaPak 150 × 3.9 mm C18 column (Waters Associates, Milford, MA, USA) preceded by a 4 mm NovaPak 20 × 3.9 mm C18 pre-column using a Waters 2695 separations module connected to a Waters 2487 absorbance detector set at 227 nm. A linear gradient was started with 100% mobile phase A pumped at a flow rate of 1 mL min^{-1}. Mobile phase A was then decreased to 70% from 10 to 11 min, with mobile phase A maintained at 70% from 11 to 16 min to remove materials slowly eluting from the column before the next injection. Subsequently, mobile phase A was increased to 100% from 16 to 17 min and maintained at 100% mobile phase A for 3 min providing a total run time of 20 min. Plasma concentrations for paclitaxel were calculated from the ratio of the area of the paclitaxel peak to the area of the *N*-cyclohexyl benzamide peak using least-squares linear regression and weighting by 1/x. Within day and between days variability measured by a coefficient of variation was < 10%. Plasma concentration profiles of normal and tumour bearing rats were compared.

Determination of paclitaxel pharmacokinetics and tumour concentration in rats using radiolabelled paclitaxel

Anaesthesia and surgery

Rats were anaesthetized with a single intraperitoneal injection of a combination of ketamine (100 mg kg^{-1}) and xylazine (2 mg kg^{-1}). The neck was cleaned with surgical disinfectant and alcohol and shaved. The right carotid artery was exposed and cannulated with sterile PE-50 tubing. A midline incision was made around the neck region and the left carotid artery was cannulated with PE-50 tubing for blood sampling. Catheters were tunnelled subcutaneously and exteriorized at the base of the neck followed by closure of incisions using surgical staples (Buehler et al 2004). The open tubing was stoppered with a fishing line. All surgery was performed under aseptic conditions. Neosporin antibiotic cream (Pfizer, Morris Plains, NJ) was applied to the wounds to prevent infection. A 45-min recovery period was given before drug administration.

Drug administration for pharmacokinetic studies

IRL 1620 was administered to tumour bearing animals (n = 4) at a dose of 3 nmol kg^{-1}, intravenously. [^{3}H]-Paclitaxel (40 μCi) was mixed with unlabelled paclitaxel so that a total dose of 5 mg kg^{-1} was injected intravenously 15 min after vehicle or IRL 1620 administration.

Drug administration for biodistribution studies

Rats were anaesthetized with ketamine (100 mg kg^{-1}) and xylazine (2 mg kg^{-1}) and allowed to stabilize for 45 min. Vehicle (0.3 mL kg^{-1}, i.v.) or IRL 1620 (3 nmol kg^{-1}) were administered and 15 min later [^{3}H]-paclitaxel (40 μCi) (Moravek Biochemicals, Brea, CA) was administered via the tail vein. Rats were sacrificed at 3 h and tumour tissue samples were collected (n = 7 in each group). It has been demonstrated that the maximal increase in tumour perfusion occurs 15 min after IRL 1620 administration (Rai et al 2004). Paclitaxel has been reported to have a Tmax value of 3 h in the tumour tissue (Klecker et al 1994). Therefore, we administered paclitaxel 15 min after IRL 1620 or vehicle injection and rats were sacrificed 3 h after paclitaxel administration.

Sample collection

Plasma was collected before vehicle or IRL 1620 administration to provide baseline values. Approximately, 0.2 mL of blood was drawn from the rats in heparinized syringes at baseline, 1, 5, 15 and 30 min and 1, 2, 4, 6, 8, 12 and 24 h. The samples were centrifuged and plasma was separated and stored at −80°C until analysis.

Analysis of [^{3}H]-paclitaxel

The concentrations of [^{3}H]-paclitaxel in the plasma and tissue samples were measured using a Beckman Coulter liquid scintillation counter (model LS 6500). Briefly, plasma was thawed and mixed with 20 mL of liquid scintillation fluid. Tissues (500 mg) were mixed in 6 mL of tissue solubilizer (TS-2; RPI, IL) overnight and then mixed with 45 mL of liquid scintillation fluid. The samples were counted and the counts were converted from dpm units to fmol mL^{-1} using the following formula:

$$\text{fmol mL}^{-1} = \text{dpm value} \times \text{decay factor} \times 2.2 \times 10^{-12}/10^{-12} \times \text{volume of sample in mL} \quad (1)$$

After conversion into fmol mL^{-1}, the pharmacokinetics of the total paclitaxel was calculated using the ratio of [^{3}H]-paclitaxel to unlabelled paclitaxel.

Pharmacokinetic analyses

Plasma paclitaxel pharmacokinetic estimates were determined using both non-compartmental and compartmental analyses as implemented in WinNonlin Pro 4.1 (Pharsight Corp, Mt. View, CA, USA).

Non-compartmental analyses

In the non-compartmental analysis, the area under the curve ($AUC_{0-\infty}$) was estimated using the trapezoidal rule to the last measurable concentration (C_{last}) and extrapolated to infinity by dividing C_{last} by the negative value of the terminal slope (λ) of the log-linear plasma concentration–time curve. The following parameters were also calculated: mean residence time (MRT_{iv}) was calculated as the reciprocal of λ, systemic clearance (CL) was calculated as the ratio of dose to $AUC_{0-\infty}$ and apparent volume distribution was calculated as the ratio of CL and λ. Plasma half-life was calculated as the product of 0.693 (natural log 2) and MRT_{iv}.

Compartmental analyses

In the compartmental analyses, a series of non-linear compartmental models were fitted to the plasma concentration–time curve data. Specifically, one-compartment, two-compartment and three-compartment models were compared. Uniform and predicted data based weighting were tested.

The final selection of the model was based on diagnostic plots (observed vs predicted and plot of residuals), Akaike Information Criteria (AIC) and Schwartz Criteria (SC). The model with a lower AIC and SC criteria was considered the final model (see Figure 3).

Blood pressure measurement

Normal and tumour bearing rats (n = 6 in each group) were anaesthetized with urethane (1.5 g kg^{-1}, intraperitoneally. The right femoral artery was cannulated (PE-50 tubing) and connected to a Gould P23 ID pressure transducer for recording the blood pressure on a Grass P7D polygraph (Grass Instrument Co., Quincy, MA) through a 7PI preamplifier. Rats were allowed to stabilize for 45 min before starting the experiment.

The effect of administration of paclitaxel (3 mg kg^{-1}, i.v.) with and without IRL 1620 (3 nmol kg^{-1}, i.v.) on blood pressure was measured up to 10 h in normal and tumour bearing rats.

Statistical analyses

All values are presented as mean ± s.e.m. and the data was analysed by one way analysis of variance followed by Duncan's test for HPLC-UV studies and by *t*-test for liquid scintillation studies. $P < 0.05$ was considered significant. The main outcome measure in these pharmacological response studies is the difference in concentration of paclitaxel in plasma.

Results

In the non-compartmental analysis, the AUC calculated for the vehicle + paclitaxel group was 9.43 ± 1.47 μg h mL^{-1} and was similar ($P > 0.05$) to that of IRL 1620-treated tumour rats. The elimination half-life was calculated as 0.14 ± 0.08 h. The clearance, calculated as dose/AUC, was estimated to be 0.56 ± 0.07 L h^{-1} kg^{-1}. The volume of distribution, calculated as clearance/K_{el}, was found to be 10.11 ± 4.18 L kg^{-1}. All the above parameters were similar in vehicle or IRL 1620-treated rats (Table 1). IRL 1620 did not affect the pharmacokinetic profile of paclitaxel in normal and tumour bearing rats (Figures 1 and 2).

The plasma concentrations of paclitaxel were calculated from the dpm counts in the plasma samples. A three-compartmental model best described the pharmacokinetics of paclitaxel. Figure 3 depicts the observed vs predicted pharmacokinetic plots for both vehicle-treated and IRL 1620-treated rats. The AUC of paclitaxel in vehicle-treated rats was 9.42 ± 3.18 μg h mL^{-1}. The steady-state volume of distribution (Vss) was 10.31 ± 4.54 L kg^{-1}. Clearance was estimated to be 0.69 ± 0.17 L h^{-1} kg^{-1}. The α t½, β t½ and γ t½ was 0.03 ± 0.01 h, 1.0 ± 0.32 h and 25.87 ± 17.81 h, respectively. The mean residence time was 27.92 ± 19.84 h. These parameters estimated in the IRL 1620-treated group were not significantly different from those in the vehicle-treated group (Table 2).

Table 1 Non-compartmental analysis of plasma pharmacokinetics of [^{3}H]-paclitaxel when administered to tumour bearing rats 15 min following vehicle (0.3 mL kg^{-1}, i.v.) or IRL 1620 (3 nmol kg^{-1}, i.v.) injection

Group	Vehicle + [^{3}H]-paclitaxel	IRL 1620 + [^{3}H]-paclitaxel
Lambda (h)	0.14 ± 0.08	0.10 ± 0.05
Cmax (μg mL^{-1})	6.73 ± 0.54	5.85 ± 0.77
$AUC_{0-\infty}$ (μg h mL^{-1})	9.43 ± 1.47	8.63 ± 0.79
Cl (L h^{-1} kg^{-1})	0.56 ± 0.07	0.60 ± 0.06
Vd (L kg^{-1})	10.11 ± 4.18	9.56 ± 2.90
Vss (L kg^{-1})	8.14 ± 2.95	8.15 ± 2.20
MRT_{inf} (h)	17.43 ± 8.13	14.48 ± 4.70

Data is presented as mean ± s.e.m., n = 4 in each group.

The effect of vehicle or IRL 1620 on [^{3}H]-paclitaxel concentration in the tumour was determined. IRL 1620 or vehicle was administered 15 min before [^{3}H]-paclitaxel administration. Rats were sacrificed 3 h after paclitaxel administration. A significant increase (308.59 ± 24.42%, $P < 0.001$) in the tumour concentration of [^{3}H]-paclitaxel was observed in rats treated with IRL 1620 compared with vehicle-treated rats (Figure 4).

The baseline mean arterial pressure (MAP) in normal rats was similar in vehicle- and IRL 1620-treated groups. In normal rats, there was no significant increase in MAP following IRL 1620 infusion for up to 10 h. There was no significant change in MAP when rats were treated with vehicle and paclitaxel or IRL 1620 and paclitaxel. The baseline MAP in tumour bearing rats was similar in vehicle- and IRL 1620-treated rats. There was no significant change in MAP when tumour bearing rats were treated with vehicle and paclitaxel or IRL 1620 and paclitaxel as well (Table 3).

Discussion

The results of non-compartmental and compartmental analyses showed that pharmacokinetics of paclitaxel in tumour bearing rats did not change when administered 15 min after IRL 1620 injection. An important finding in this study was that the pharmacokinetic profile of paclitaxel was similar in both normal and tumour bearing rats, indicating that the tumour model used in this study did not compromise paclitaxel disposition in the body (Figure 1). In this study, a three-compartmental model best described the plasma pharmacokinetics of paclitaxel. This model suggests that paclitaxel is distributed to various organs whether the blood perfusion in the organs is high, medium or low. IRL 1620 administration did not change the distribution of paclitaxel. The plasma pharmacokinetic parameters, generated by the three-compartment model, displayed comparable clearances, volumes of distribution and absorption, distribution and elimination

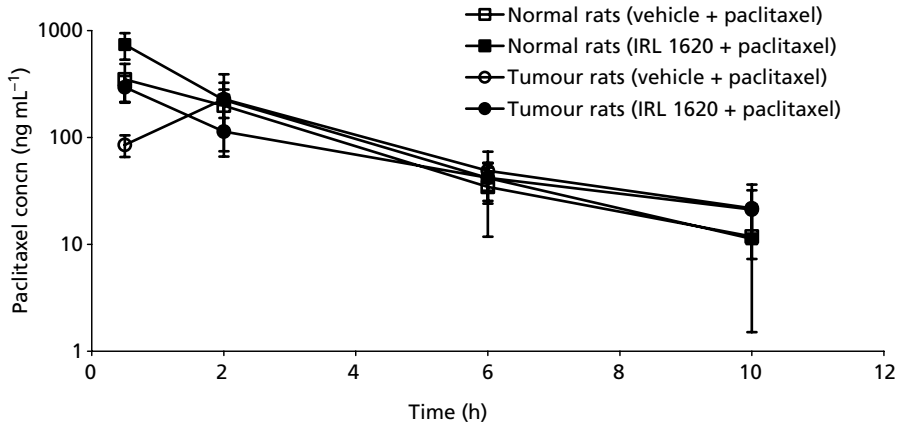

Figure 1 Effect of vehicle (0.3 mL kg^{-1}, i.v.) or IRL 1620 (3 nmo kg^{-1}, i.v.) on plasma pharmacokinetics of paclitaxel as determined by HPLC analysis in normal and tumour bearing rats. Data is presented as mean ± s.e.m., n = 4 in each group.

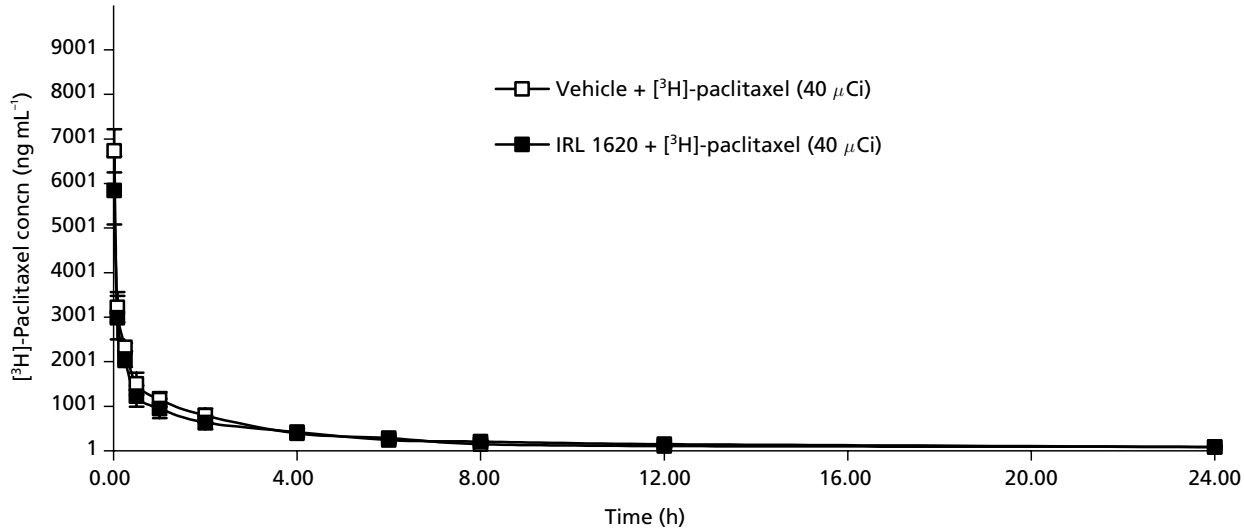

Figure 2 Effect of vehicle (0.3 mL kg^{-1}, i.v.) or IRL 1620 (3 nmol kg^{-1}, i.v.) on plasma pharmacokinetics of [^{3}H]-paclitaxel in tumour bearing rats as determined by liquid scintillation counting. Data is presented as mean ± s.e.m., n = 4 in each group.

half-lives for the groups treated with vehicle and IRL 1620. However, we have shown earlier that IRL 1620 increases tumour blood perfusion and tumour paclitaxel concentration (Rai et al 2004). Results of this study also confirm that IRL 1620 injected 15 min before paclitaxel administration significantly increased the tumour concentration of paclitaxel. Therefore, IRL 1620 selectively increases tumour perfusion and paclitaxel concentration without significantly altering the pharmacokinetic profile of paclitaxel.

The pharmacokinetics of IRL 1620 has not been studied extensively. However, it has been shown that endothelins have a short half-life of 7–8 min (Rubin & Levin 1994). As an analogue of ET-1, IRL 1620 may have a similar half-life. The duration of effect of IRL 1620 was about 2 h post administration, which is similar to that of ET-1.

Preclinical and clinical studies have shown that paclitaxel displays non-linear pharmacokinetics in the plasma when administered at low doses (Huizing et al 1993; Hurwitz & McGuire 1994; Sonnichsen & Relling 1994; Gianni et al 1995; Sparreboom et al 1996a, b). With an infusion of up to 24 h duration, most studies have fitted a first-order two-compartment model to describe paclitaxel disposition in the body (Longnecker et al 1987; Wiernik et al 1987a, b; Brown et al 1991; Rowinsky et al 1993). However, with enhanced assay sensitivity a three-compartment model was detected (Huizing et al 1993; Schiller et al 1994; Sonnichsen & Relling 1994). A two-compartment model does not adequately describe the discrepancy between the gradual increase in intra-infusion concentration and the rapid decrease once infusion is stopped. Thus, distribution may be saturable when admi-

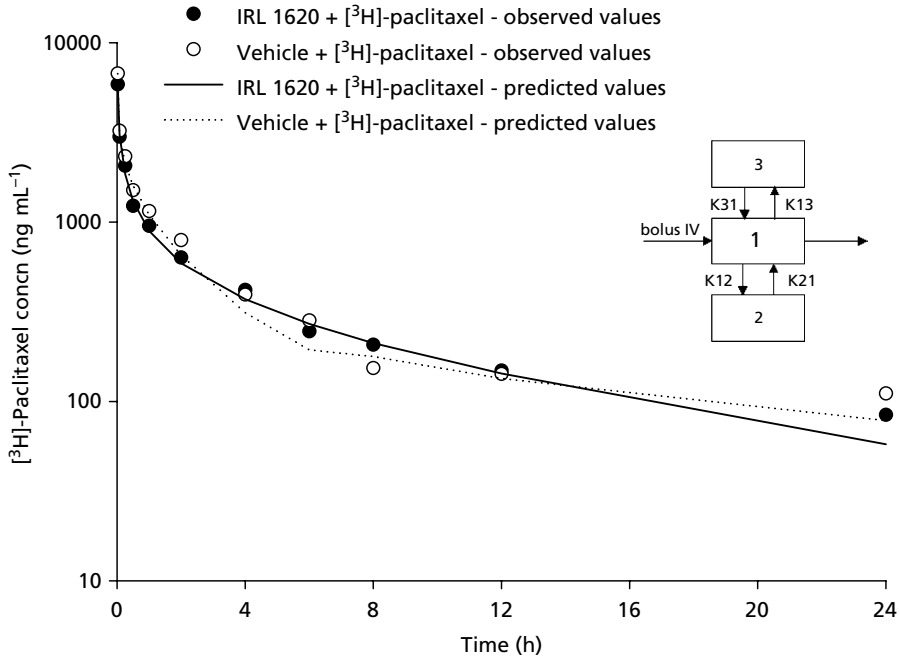

Figure 3 Model fit was assessed by overlaying predicted curves with the observed [^{3}H]-paclitaxel as a function of time. Inset shows a three-compartment intravenous bolus model with no lag time and first-order elimination.

Table 2 Three-compartmental analysis of plasma pharmacokinetic data of [^{3}H]-paclitaxel when administered to tumour bearing rats 15 min following vehicle (0.3 mL kg^{-1}, i.v.) or IRL 1620 (3 nmol kg^{-1}, i.v.) injection

Group	Vehicle + [^{3}H]-paclitaxel	IRL 1620 + [^{3}H]-paclitaxel
$AUC_{0-\infty}$ (μg h mL^{-1})	9.42 ± 3.18	7.25 ± 0.76
Cl (L h^{-1} kg^{-1})	0.69 ± 0.17	0.72 ± 0.09
MRT (h)	27.92 ± 19.84	10.58 ± 3.20
Vss (L kg^{-1})	10.31 ± 4.54	7.28 ± 1.79
α $t_{½}$ (h)	0.03 ± 0.01	0.04 ± 0.01
β $t_{½}$ (h)	1.00 ± 0.32	0.84 ± 0.32
γ $t_{½}$ (h)	25.87 ± 17.81	9.42 ± 2.59
K_{10} (h^{-1})	3.14 ± 1.34	1.72 ± 0.57
K_{12} (h^{-1})	56.47 ± 27.69	34.93 ± 23.26
K_{13} (h^{-1})	5.71 ± 3.37	3.92 ± 1.88

Data is presented as mean ± s.e.m., n = 4 in each group.

nistered at high doses or as short infusions (Sonnichsen & Relling 1994).

The choice of an analytical method determines the sensitivity with which paclitaxel can be measured. It also dictates number of compartments that can be used to describe the pharmacokinetics of paclitaxel. It has been found that paclitaxel follows a three-compartment model with three exponential decay curve. The half-lives of these three phases were 0.2 h (α-phase), 1.9 h (β-phase) and 20.7 h (γ-phase), indicating that the drug circulates for a prolonged time (Huizing et al 1993). Our results are in accordance with published literature and follow a three-compartment model with a three exponential decay curve.

To achieve high concentrations of the drug at the tumour site selectively, we have used the vasodilatory properties of IRL 1620 and transiently increased the concentration of paclitaxel in the tumour. We define tumour targeting to mean that paclitaxel reaches higher concentrations in the tumour with the pre-administration of IRL 1620 than without. Previous studies in our laboratory have shown that IRL 1620 increases perfusion to the tumour at 15 min. Therefore, [^{3}H]-paclitaxel was administered 15 min after IRL 1620. Previous autoradiographic studies have shown that [^{3}H]-paclitaxel distributes in high concentrations in a variety of tissues (Lesser et al 1995). Specifically, high concentrations were found in the liver, kidney, spleen, lung and heart and the maximum tissue concentration of paclitaxel in the tumours was found to be at 3 h (Klecker et al 1994). An increase in tumour concentration of [^{3}H]-paclitaxel with the administration of IRL 1620 indicates that ET_B receptor stimulation in the tumour can increase the delivery of chemotherapeutic drugs selectively to the tumour.

Paclitaxel is known to cause orthostatic hypotension clinically (Jerian et al 1993; Vassilomanolakis et al 1998). However, the change in blood pressure appears to be dose limiting (Ekholm et al 1997). Studies suggest that paclitaxel treatment reduces blood pressure variability but does not impair cardiac autonomic function (Ekholm et al 1997). IRL 1620 causes a very transient decrease in

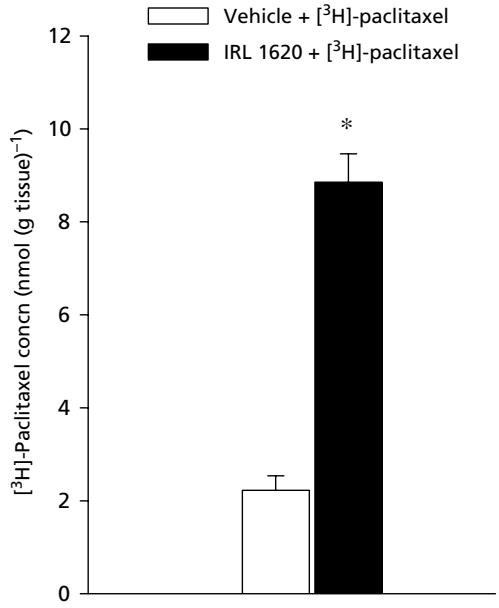

Figure 4 Effect of vehicle (0.3 mL kg^{-1}, i.v.) or IRL 1620 (3 nmol kg^{-1}, i.v.) on [^{3}H]-paclitaxel concentration in tumour tissue of tumour bearing rats. Data is presented as mean ± s.e.m., n = 7 in each group. *$P < 0.001$ vs vehicle-treated group.

blood pressure and therefore co-administration with paclitaxel could potentially increase the risk of hypotension. However, we found that paclitaxel did not aggravate the change in pressure induced by IRL 1620. This could be partially due to the non-overlapping administration of the drugs, as well as to the low dose of paclitaxel administered. Paclitaxel was given 15 min after IRL 1620, by which time the depressor response to IRL 1620 was attenuated. Thus, the potential cardiovascular adverse effects of this combination of drugs were minimized by optimal timing of drug administration.

Overall, these results suggest that the use of IRL 1620 did not affect the pharmacokinetics of paclitaxel, indicating that the safety of paclitaxel does not change due to the administration of IRL 1620. Thus, IRL 1620 could potentially be used to improve paclitaxel efficacy and allow for appropriate dose titration to minimize its severe toxicities. Our results show that the pharmacokinetic profile of paclitaxel in normal rats was similar to that in tumour bearing rats, indicating that paclitaxel disposition is not altered by the tumour model system.

From a drug development perspective, several trials are in progress where a neutralizing antibody against vascular endothelial growth factor (VEGF) in combination with standard chemotherapy drugs produced an increase in survival (5 months) in patients with colorectal cancer (Hurwitz et al 2004). It is not clearly established whether combined therapy yields maximal benefit because the drugs act through two separate mechanisms, one destroying cancer cells and the other endothelial cells. Chemotherapy and radiation therapy may also have anti-angiogenic effects or cancer cells may express receptor for VEGF (Hicklin & Ellis 2005). However, it is also possible that destroying the vasculature would decrease the delivery of oxygen and chemotherapeutic agents to the solid tumour and may render them ineffective. It has been proposed that anti-angiogenic agents can normalize the abnormal tumour vasculature, leading to more efficient delivery of oxygen and chemotherapeutic agents to the tumour (Jain 2005). We provide evidence of a novel approach of using IRL 1620, an ET_B receptor agonist, to increase tumour perfusion and delivery of paclitaxel to the tumour tissue (Rai & Gulati 2003; Rai et al 2004). We propose that a combination of ET_B receptor agonist with standard chemotherapy or radiotherapy should increase their effectiveness.

Conclusions

It is concluded that IRL 1620 enhances delivery of paclitaxel selectively to the tumour but does not affect plasma pharmacokinetics of paclitaxel or blood pressure in breast tumour bearing rats. These studies provide a background for future efficacy studies. The results suggests that IRL 1620 can potentially increase paclitaxel efficacy without affecting its pharmacokinetics and cardiovascular effects.

Table 3 Effect of vehicle (0.3 mL kg^{-1}, i.v.) or IRL 1620 (3 nmol kg^{-1}, i.v.) and paclitaxel on mean arterial pressure in tumour bearing rats

	Group	Mean arterial pressure (mmHg)				
		Baseline	0.5 h	2.0 h	6.0 h	10.0 h
Normal rats	Vehicle + paclitaxel	151.17 ± 8.75	145.83 ± 4.22	147.92 ± 12.18	136.25 ± 13.18	133.75 ± 15.66
	IRL 1620 + paclitaxel	143.75 ± 5.79	155.42 ± 10.03	159.58 ± 5.71	139.17 ± 14.62	132.50 ± 8.67
Tumour rats	Vehicle + paclitaxel	142.5 ± 6.47	151.25 ± 8.29	136.67 ± 11.65	142.92 ± 6.50	144.59 ± 6.36
	IRL 1620 + paclitaxel	139.58 ± 1.74	148.33 ± 10.04	137.92 ± 1.93	133.33 ± 2.50	132.50 ± 3.76

Paclitaxel (3 mg kg^{-1}) was administered 15 min after IRL 1620 (3 nmol kg^{-1}, i.v.) or vehicle (0.3 mL kg^{-1}, i.v.) and blood pressure was monitored for 10 h. Data is presented as mean ± s.e.m., n = 6 in each group.

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