Enantioselectivity of thalidomide serum and tissue concentrations in a rat glioma model and effects of combination treatment with cisplatin and BCNU

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Abstract

Thalidomide is currently under evaluation as an anti-angiogenic agent in cancer treatment, alone and in combination with cytotoxic agents. Thalidomide is a racemate with known pharmacologic and pharmacokinetic enantioselectivity. In a previous study with thalidomide combination chemotherapy, we found evidence of anti-tumour synergy. In this study, we examined whether the synergy involved altered pharmacokinetics of thalidomide enantiomers. Adult female F344 rats were implanted with 9L gliosarcoma tumours intracranially, subcutaneously (flank), or both. Effectiveness of oral thalidomide alone, and with intraperitoneal BCNU or cisplatin combination chemotherapy, was assessed after several weeks treatment. Presumed pseudo steady-state serum, tumour and other tissues, collected after treatment, were assayed for *R*- and *S*-thalidomide by chiral HPLC. Both serum and tissue concentrations of *R*-thalidomide were 40–50% greater than those of *S*-thalidomide. Co-administration of BCNU or cisplatin with thalidomide did not alter the concentration enantioselectivity. Poor correlation of concentration with subcutaneous anti-tumour effect was found for individual treatments, and with all treatments for intracranial tumours. The consistency of the enantiomer concentration ratios across treatments strongly suggests that the favourable anti-tumour outcomes from interactions between thalidomide and the cytotoxic agents BCNU and cisplatin did not have altered enantioselectivity of thalidomide pharmacokinetics as their basis.

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Introduction

Thalidomide, originally marketed as a safe sedative/hypnotic, became the drug of choice for nausea in early pregnancy during the 1950s. In addition to teratogenesis, for which it subsequently became infamous, thalidomide has progressively become recognized to have a variety of potentially salutary actions, notably immunomodulation and anti-angiogenesis, that make it an attractive candidate for adjunctive treatment of certain types of cancers (Matthews & McCoy 2003; Sleijfer et al 2004).

Thalidomide is made and used as a racemic mixture. The *R*- and *S*-thalidomide enantiomers have qualitatively different pharmacological effects (Eriksson et al 2001; Teo et al 2004). In essence, *R*-thalidomide has sedative properties and *S*-thalidomide has immunomodulatory and teratogenic properties. Moreover, the enantiomers also have quantitatively different pharmacokinetics. Overall, blood concentrations of *R*-thalidomide exceed those of *S*-thalidomide due, essentially, to a greater apparent total body clearance of *S*-thalidomide (Eriksson et al 2000, 2001). However, as both enantiomers will rapidly racemize in-vivo, the potential advantage of using enantiopure *S*-thalidomide for achieving greater pharmacological selectivity could, thereby, be negated (Eriksson et al 2000, 2001).

It has been hypothesized that thalidomide and chemotherapy might synergize, perhaps due to pharmacodynamic or pharmacokinetic interactions (Ding et al 2002; Chung et al 2004; Teo et al 2004). We have been exploring thalidomide adjunctive chemotherapy with cisplatin and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, carmustine) in an experimental model of 9L gliosarcoma cells transplanted into the syngeneic adult female Fischer 344 rat. We have previously demonstrated improved anti-tumour effects from such combination

therapy (Murphy et al submitted). In this report, we consider whether the interactions between thalidomide and cytotoxic agents on tumour growth had an overtly enantioselective basis. To do this, we measured the concentrations of R- and S-thalidomide in serum, tumours and other tissues, and determined whether they were altered by concomitant administration of the cytotoxic agents, BCNU and cisplatin.

Materials and Methods

Animals

Female Fischer 344 rats, obtained from the Animal Resources Centre, Perth, Australia, were used. There was no basis to presuppose a sex difference in response to cytotoxic agents or thalidomide, but out of prudence, only female animals were used throughout. Moreover, females, being of smaller body weight than males, allowed smaller amounts of chemotherapeutic drugs and thalidomide to be used.

All animal procedures were performed with protocols approved by the institutional animal care and ethics committee, and in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The method of euthanasia approved by this committee was exsanguination under general anaesthesia.

Experimental model of tumour growth

The 9L rat gliosarcoma cell line used was provided by the University of San Francisco Neurosurgery Department. The cells were maintained in culture in Basal Medium Eagles media supplemented with 10% fetal calf serum and L-glutamine ($398\,\mu g\,mL^{-1}$) (Barker et al 1973). The cells were subcultured twice a week. In preparation for an experiment, an adult female Fischer 344 rat was injected subcutaneously with 5×10^6 cells in both flanks, two weeks before the planned experiment; the 9L gliosarcoma cell line is syngeneic with this strain of rat. The rat was then euthanized; tumours were removed and cut into 1.0-mm cubes immediately before implantation into the skull or flanks of the experimental rats, using previously described techniques (Kimler 1994).

Under general anaesthesia, aseptic surgical techniques were used to implant either or both of subcutaneous flank, and intracranial, tumours as previously described (Murphy et al submitted). The rats were monitored for body weight, tumour progression, feeding ability, external appearance, locomotion, neurological signs and signs of pain or distress also as described previously. The rats were euthanized when the relevant endpoint (duration of exposure, size of tumour or behavioural endpoint) was reached according to the particular protocol.

Drug administration

In various experiments, drug treatments consisted of BCNU (2.5, 5, 7, 10 or $20\,mg\,kg^{-1}$, i.p.), cisplatin (0.01, 0.02 or $1\,mg\,kg^{-1}$, i.p.) or thalidomide (1% w/w in food), and combinations of thalidomide with BCNU or cisplatin.

Blood and tissue collection

Blood and tissues were collected immediately after euthanasia. Blood samples were obtained by cardiac puncture. In experiments with intracranial tumours, the brain was removed whole, the tumour was dissected and weighed, snap frozen and stored at $-80°C$ pending analysis. On some occasions, non-tumour brain tissue samples adjacent to, and contralateral to, the tumour were also collected at the same time. The flank tumour and, on some occasions, heart, lung, liver, kidney and skeletal muscle tissues, were also collected at the same time and processed the same way. Groups of 8 rats were normally used, but the final number available for sample collection varied due to attrition during the course of the experiments. The combinations of doses of cytotoxic agents and samples collected varied between experiments.

Enantiomeric resolution and quantitation

Chiral HPLC of thalidomide was performed on chemically and chirally stabilized serum and tissue extracts as described previously (Murphy-Poulton et al 2006). Briefly, thalidomide enantiomers and phenacetin internal standard were extracted with diethyl ether and separated on vancomycin chiral stationary phase using a mobile phase of 20% ACN, $HCOONH_4$ (5 mM, pH 5.5) at a flow rate of $1.0\,mL\,min^{-1}$, with UV detection at 220 nm. Over a thalidomide concentration range of 0.1–$20\,\mu g\,mL^{-1}$, assay precision was 1–5% (CV) for both enantiomers, and calibration curves were linear with all correlation coefficients being > 0.99. The estimated limit of quantification for both enantiomers was $0.05\,\mu g\,mL^{-1}$ with 0.2–0.6 mL serum or equivalent tissue samples.

Data analysis, assumptions and calculations

The available data consisted of single-point estimates of serum and relevant tissue R- and S-thalidomide concentrations after its ingestion in food at presumed pseudo-steady state. The systemic availability of oral thalidomide administered by gavage in rabbits exceeds 80% (Schumacher, Blake & Gillette 1968) but it has not, to our knowledge, been estimated in the rat. The terminal half-life of thalidomide in the rat is 3–4 h (Huang et al 2005; Yang et al 2005); thus, ingestion of thalidomide in food for at least 1 week, as in these experiments, can be assumed to produce pseudo-steady state conditions within several days even if allowing for differences in establishment and feeding patterns of individual animals. Thalidomide pharmacokinetics in man are known to be complex due to interconversion between enantiomers in-vivo; it is assumed that this occurs also in other species. Although the mean thalidomide total body clearance is inversely proportional to the steady-state blood concentration, interconversion between enantiomers makes it impossible to determine the true mean total body clearance of each enantiomer, independently, from the available data. This is further complicated by the lack of information about the relative systemic availability of the thalidomide enantiomers when administered in food. Being measured in the same biofluid samples, and with

the assumption that daily food intake is essentially constant before sampling, the relative serum concentrations of *R*- and *S*-thalidomide immediately after euthanasia will approximately reflect their relative apparent clearances.

Tissue drug concentrations are regulated by local tissue solubility/binding and clearance, as well as the prevailing serum (or, more precisely, unbound plasma) concentration. Thalidomide is hydrolysed in plasma and extra-hepatic tissues (Schumacher et al 1968, 1970) but it is not known whether extra-hepatic tissue clearance or distribution are also enantioselective. The sampled tumour tissue thalidomide concentration is a mean concentration and, as this is not necessarily the same as the unbound concentration at the tumour cell site of action, it may limit the relationship with biological effect. However, it was not feasible to perform studies with tissue and serum ultrafiltrates to calculate unbound concentrations due to limitations of assay sensitivity from the (small) tumour specimens available. Moreover, tissues may contain some residuum of blood-borne drug (Khor & Mayersohn 1991; Khor et al 1991). Preliminary calculations with and without correction for the notional blood-borne thalidomide residues were made, however, and found not to materially influence the results.

Despite the assumptions and limitations, the study provided a reasonable first estimate of the tissue distribution of the thalidomide enantiomers when used alone and combined with cytotoxic agents.

Enantioselectivity of thalidomide clearance and distribution was tested at several levels. Differences in *R*- and *S*-thalidomide concentrations were initially evaluated using Student's *t*-test for paired data but, due to inter-subject variability, these were an inefficient test of pharmacokinetic enantioselectivity. Enantioselective clearance or distribution was better evaluated by testing the relevant respective *S/R* enantiomeric ratio against a value of unity using Student's one-sample *t*-test. Where appropriate, comparison of the respective *S/R* enantiomeric ratio across treatment groups was tested by confluence of 95% confidence intervals and one-way analysis of variance (with Tukey's post test for multiple comparisons). Thalidomide concentration–tumour response correlation was examined by inspection for linear or sigmoidal tendency, and the relevant equation was fitted to the data by the method of least squares. Summary data are presented as mean ± s.e.m. or ± 95% confidence interval, as appropriate.

Results and Discussion

Serum concentrations and clearance

Serum concentrations of *R*- and *S*-thalidomide were highly correlated, with those of *R*-thalidomide always exceeding those of *S*-thalidomide across all treatments (Figures 1 and 2). Across all experiments, the mean (and 95% CI) *S/R* enantiomer concentration ratio of thalidomide serum concentrations was 0.53 (0.51–0.56) for treatment with thalidomide alone, 0.56 (0.54–0.58) for BCNU combination treatments (up to 10 mg kg^{-1}; however, for combination treatment with BCNU

20 mg kg^{-1}, the mean value, 0.73, was significantly greater than the other treatments) and 0.55 (0.51–0.58) for cisplatin combination treatments (all less than unity, $P < 0.0001$). This is consistent with a greater apparent mean total body clearance of *S*- than *R*-thalidomide, and was not systematically altered by either BCNU or cisplatin combination treatment.

Distribution into tumour tissues

Tumour concentrations of *R*-thalidomide consistently exceeded those of *S*-thalidomide across all treatments (Figures 1 and 2). The mean (and 95% CI) *S/R* enantiomer concentration ratios were, respectively, 0.55 (0.51–0.60), 0.60 (0.58–0.61) and 0.57 (0.51–0.62) and 0.59 (0.52–0.61) for thalidomide alone, BCNU and cisplatin combination treatments (all less than unity, $P < 0.0001$). Hence, there was a greater relative concentration of *R*- than *S*-thalidomide in tissues of both tumour sites, and this was not systematically altered by BCNU or low-dose cisplatin in the combination treatments.

Across all experiments, the tumour thalidomide concentrations of both enantiomers were related, overall, to the corresponding serum concentrations without any systematic effect of BCNU or low-dose cisplatin co-treatment (Figures 1 and 2). However, for co-treatment with cisplatin (1 mg kg^{-1}), the concentrations of both enantiomers in both the subcutaneous flank and intracranial tumours, relative to serum, were nearly double those of the other treatments. However, the volumes of the co-treated tumours were much smaller, averaging approximately one-quarter (subcutaneous flank) and approximately one-third (intracranial) that of the mono-treated, so it is difficult to interpret this observation without further information on tumour density and composition.

Distribution into non-tumour tissues

The thalidomide enantiomer concentration ratio between whole blood and serum in the same samples measured after treatment with thalidomide alone and with BCNU 7 mg kg^{-1} did not significantly differ from unity for either enantiomer after either treatment. The respective *R*- and *S*-thalidomide blood:serum distribution coefficients (and 95% CI) were 0.85 (0.54–1.17) and 0.90 (0.58–1.22) after thalidomide alone and 0.91 (0.81–1.02) and 0.98 (0.86–1.10) after thalidomide + BCNU.

Thalidomide enantiomer concentrations in various tissues and serum, alone and with cisplatin (0.01 and 0.02 mg kg^{-1}), are shown in Figure 3. Although the concentrations varied among tissues, the concentration of *R*-thalidomide in each tissue consistently exceeded that of *S*-thalidomide, in a similar ratio to that found for serum and tumour tissue. Overall, tissue:serum distribution coefficients for *R*- and *S*-thalidomide tended to be greater for *S*- than *R*-thalidomide, without any systematic influence of low-dose cisplatin in the combination treatments (e.g. Table 1; Figure 4). Moreover, in experiments with intracranial tumours, *R*-thalidomide concentrations in brain adjacent and contralateral to the intracranial tumour always exceeded those of *S*-thalidomide ($P < 0.001$) with or without cisplatin (Figure 5) or BCNU (data not shown).
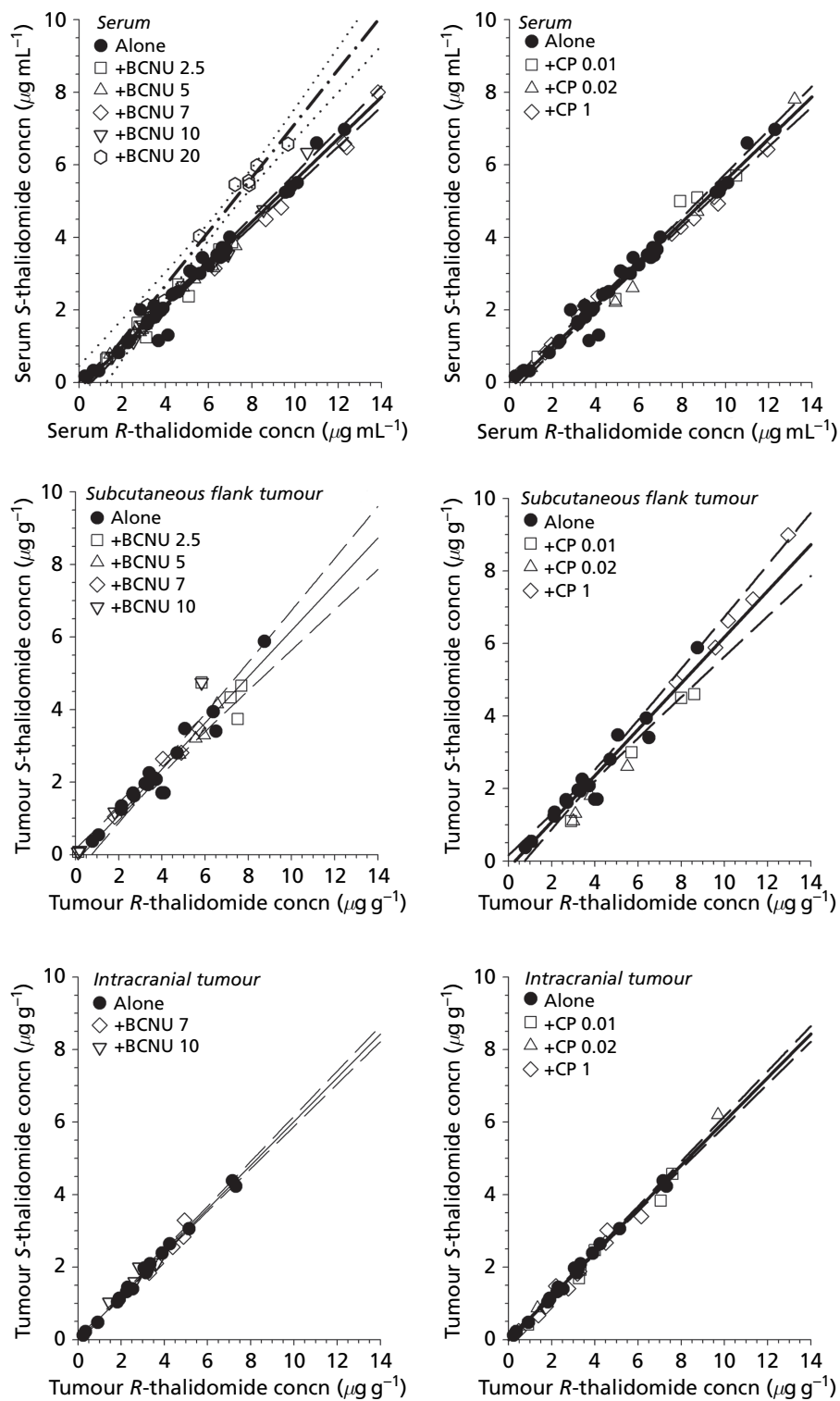
Figure 1   Serum and tumour concentrations of *R*- and *S*-thalidomide found when thalidomide was administered to rats alone, with BCNU 2.5, 5, 7, 10 or 20 mg kg^{-1}, or with cisplatin 0.01, 0.02 or 1 mg kg^{-1}. Each point is derived from a single subject. In each panel, the least squares linear regression with 95%CI is shown for thalidomide alone. In the top left panel, the least squares linear regression with 95%CI is also shown for thalidomide + BCNU 20 mg/kg; it is clear that only these data systematically lie outside the 95% CI for thalidomide alone.
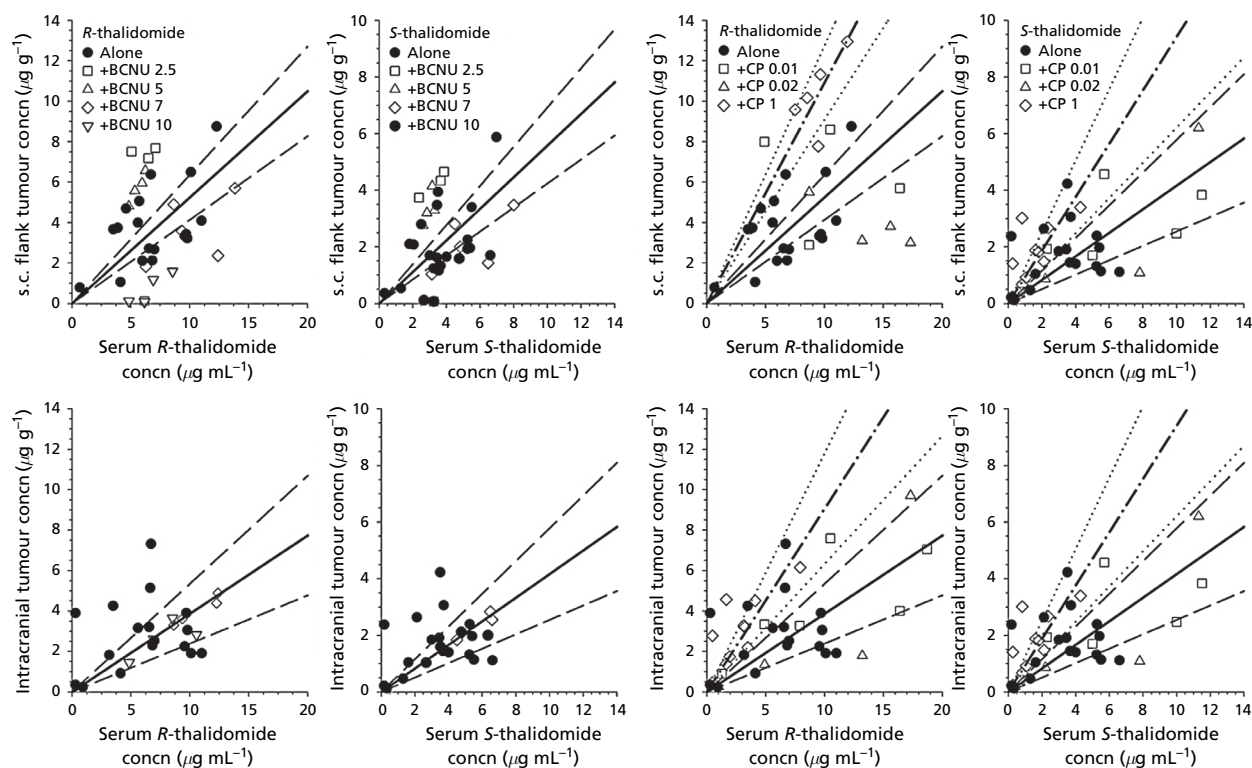
Figure 2 Serum and subcutaneous flank (upper panels) or intracranial (lower panels) tumour concentrations of *R*- and *S*-thalidomide found when thalidomide was administered to rats alone, with BCNU 2.5, 5, 7, 10 or 20 mg kg^{-1} or with cisplatin 0.01, 0.02 or 1 mg kg^{-1}. Each point is derived from a single subject. In each panel, the least squares linear regression forced through the origin with 95%CI is shown for thalidomide alone. There is no convincing demonstration of systematic deviation for the data with BCNU co-treatments, but it is clear that the data for thalidomide + cisplatin at 1 mg kg^{-1} systematically lie outside the 95% CI for thalidomide alone.

Thalidomide concentration–response relationships

The anti-tumour responses of subcutaneous flank or intracranial tumours were not significantly correlated with serum or subcutaneous flank or intracranial tumour concentrations of either thalidomide enantiomer from rats treated with any one treatment (data not shown). However, when the subcutaneous flank tumour data from thalidomide alone and with cisplatin (1 mg kg^{-1}) (Figure 6) or BCNU (5 mg kg^{-1}) (data not shown) were combined, apparently sigmoidal relationships between tumour response and tumour and serum concentrations of both thalidomide enantiomers were discernible but, it is emphasized, only in the presence of cisplatin and each other. From these, the respective estimated EC50 values for *S*-thalidomide, approximately 4.0 and 6.2 μg g^{-1}, were smaller than those of R-thalidomide, approximately 6.8 and 9.9 μg g^{-1}. Analogous correlation was not apparent for intracranial tumours (data not shown).

Optimal use of thalidomide or its analogues in cancer treatment requires an understanding of its mechanism of action, both with and without concurrent chemotherapy, mainly to retain or enhance anti-tumour activity while eliminating thalidomide toxicity. In this context, the activity of the thalidomide enantiomers ostensibly presents a vital area of research, since it is possible that an enantioselective pharmacokinetic drug interaction could produce enhanced activity,

perhaps through enhanced tumour concentration of the pharmacologically preferred enantiomer. This, at least hypothetically, could succeed, whereas the occurrence of metabolic racemization would presently seem to preclude use of a preferred enantiopure thalidomide preparation.

In a previous study with thalidomide combination chemotherapy we found evidence of anti-tumour synergy (Murphy et al submitted). Thus, in this preliminary analysis, we examined whether the synergy may have altered pharmacokinetics of thalidomide enantiomers as a basis. Although we found abundant evidence for enantioselectivity of thalidomide pharmacokinetics, we found no convincing evidence for altered enantioselectivity of thalidomide clearance or tissue distribution due to concurrent cytotoxic agents.

Based on serum and tissue samples obtained at only one time point (i.e. immediately after euthanasia) and subject to some assumptions about pseudo-steady state conditions pertaining, a clear demonstration of thalidomide pharmacokinetic enantioselectivity was obtained. While it is possible that the procedure for euthanizing the rats altered the values, and there is no way of determining this, the patterns were very consistent throughout a long series of experiments.

First, the relative apparent mean total body clearance in the rat of *S*-thalidomide was found to be some 40–50% greater than that of *R*-thalidomide; this is similar to findings
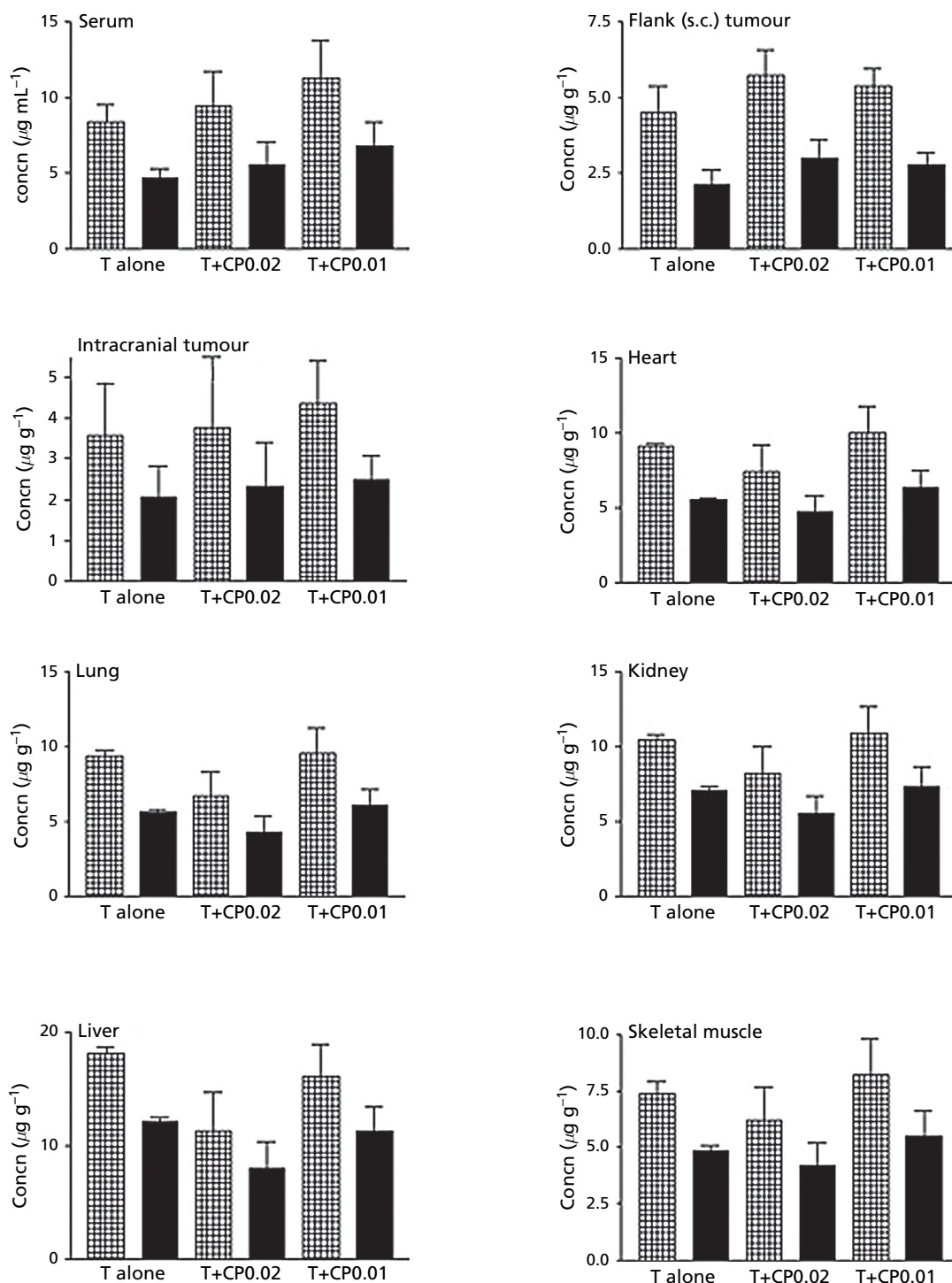
Figure 3   Mean (± s.e.m.) concentrations of *R*- and *S*-thalidomide (respectively, checked and solid bars) in serum, subcutaneous flank tumours, intracranial tumours, heart, lung, kidney, liver and skeletal muscle when thalidomide was administered to rats alone (T alone), and with cisplatin 0.01 mg kg^{-1} (T + CP0.01) or cisplatin 0.02 mg kg^{-1} (T + CP0.02).

in healthy human subjects with the administration of the separate enantiomers (Eriksson et al 2000). Second, the concentrations of *R*-thalidomide were 40–50% greater than those of *S*-thalidomide in virtually every tissue examined, including

tumour tissue. Third, the relative tissue:serum distribution coefficients indicated a marginally (10–20%) greater relative uptake of *S*-thalidomide than *R*-thalidomide for all tissues examined, except for tumour wherein the relative uptake of

Table 1 Enantioselectivity of thalidomide distribution determined from the *R/S* ratio of the respective enantiomer tissue/serum distribution coefficients for thalidomide treatment alone, and with 0.01 and 0.02 mg kg^{-1} doses of cisplatin

Treatment group	Heart	Lung	Kidney	Muscle	Liver	s. c. Tumour
All	0.92	0.91	0.85	0.87	0.85	1.18
	[0.87–0.97]	[0.86–0.96]	[0.81–0.89]	[0.84–0.90]	[0.80–0.90]	[1.06–1.30]
	$P = 0.0023$	$P = 0.0015$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.0063$
T alone	0.91	0.91	0.84	0.84	0.81	1.26
	[0.85–0.98]	[0.84–0.98]	[0.79–0.89]	[0.79–0.90]	[0.76–0.86]	[1.00–1.52]
	$P = 0.017$	$P = 0.020$	$P = 0.0003$	$P = 0.0003$	$P = 0.0002$	$P = 0.048$
T+CP0.01	0.87	0.86	0.81	0.89	0.86	1.15
	[0.73–1.00]	[0.76–0.97]	[0.70–0.92]	[0.84–0.94]	[0.71–1.01]	[1.03–1.28]
	$P = 0.047$	$P = 0.024$	$P = 0.005$	$P = 0.004$	$P = 0.061$	$P = 0.028$
T+CP0.02	0.96	0.96	0.89	0.87	0.88	1.09
	[0.87–1.06]	[0.85–1.06]	[0.82–0.95]	[0.82–0.93]	[0.78–0.97]	[0.70–1.47]
	$P = 0.40$	$P = 0.37$	$P = 0.004$	$P = 0.001$	$P = 0.017$	$P = 0.51$

T, thalidomide; CP0.01 and CP0.02, cisplatin 0.01 and 0.02 mg kg^{-1}, respectively. Mean [a 95% CI], and significance when tested against a value of unity (Student's one-sample *t*-test).

R-thalidomide was 10–20% greater than *S*-thalidomide. A higher total tissue uptake/distribution of *S*-thalidomide is consistent with the finding of a greater mean apparent volume of distribution/bioavailable fraction of dose (V/F ratio) of *S*-thalidomide in healthy human subjects with the administration of the separate enantiomers. Recent work performed with CaCo cells indicates that neither thalidomide enantiomer is a substrate for P-glycoprotein transport (Zhou et al 2005; Zimmermann et al 2006); although the interaction of thalidomide and other transporter systems is not yet known, enantioselectivity of tissue influx/efflux may not explain the observations.

Next, the effect of combining treatment of thalidomide with the chemotherapeutic agents BCNU and cisplatin was assessed. As this might have involved differential alterations to clearance and distribution of the enantiomers, the relative respective enantiomeric ratios were examined. Although the serum concentration ratio (and thus relative apparent clearance or distribution) of enantiomers was altered by BCNU 20 mg kg^{-1}, this was an aberration in that this dose also caused excessive toxicity and was not investigated beyond the serum thalidomide concentrations. Moreover, cisplatin at 1 mg kg^{-1} produced a greater tumour tissue:serum distribution of thalidomide, along with a markedly decreased tumour size, but the effects were similar for both enantiomers. Overall, therefore, there was no enantiomeric difference regardless of treatment (i.e. thalidomide alone, or with cisplatin or BCNU), or dose of cisplatin or BCNU, suggesting that enhanced anti-tumour activity of combination treatment was not related to alterations in the thalidomide enantiomeric ratio. Indeed, the evidence in our previous report strongly suggested that the effects were related to alterations in vascular growth factors (Murphy et al submitted).

Finally, some preliminary attempts were made to explore thalidomide concentration–tumour response relationships. Poor correlations were found for individual treatments, and it was not until data were combined for thalidomide alone and with high dose cisplatin that a sigmoidal relationship was apparent. From this, *S*-thalidomide produced a lower response EC50 than *R*-thalidomide

for subcutaneous tumour response. However, these values are not absolute as they were always obtained in the presence of the alternative enantiomer and, as thalidomide monotherapy was largely ineffectual, they also required the presence of cisplatin co-treatment to dramatically increase the response. Although the ratio of responses in treated and control groups (expressed as %) is commonly used as an outcome variable, it can, and did, generate values greater than 100% due to vagaries in both groups. Values greater than 100% were observed when animals were, in particular, treated with thalidomide alone. Moreover, as pointed out above, the tumour concentrations are mean concentrations, and these may not be equal to those at the cellular site of action. Because of the consistency of the enantiomeric ratio of thalidomide concentrations in serum and tumour tissues, the potential activity of thalidomide metabolites, the lack of a true site of action concentration, and in the absence of studies with enantiopure thalidomide, it is reasonable to conclude that a more formal concentration–response relationship analysis is unlikely to produce a more insightful conclusion than that from the preliminary analysis performed above.

Conclusion

Inspection of the enantiomeric concentration ratios and distribution coefficients demonstrated that enantioselectivity in thalidomide pharmacokinetics in the rat pertains after administration of *rac*-thalidomide in a manner consistent with that already described in man. This, coupled with the known differences in the pharmacology of the thalidomide enantiomers, suggests that the design of future mechanistic or explanatory studies of thalidomide pharmacotherapy should include quantitative techniques that separate the enantiomers. Nonetheless, observed favourable anti-tumour outcomes from interactions between thalidomide and the cytotoxic agents BCNU and cisplatin would seem not to involve altered enantioselectivity of thalidomide pharmacokinetics.
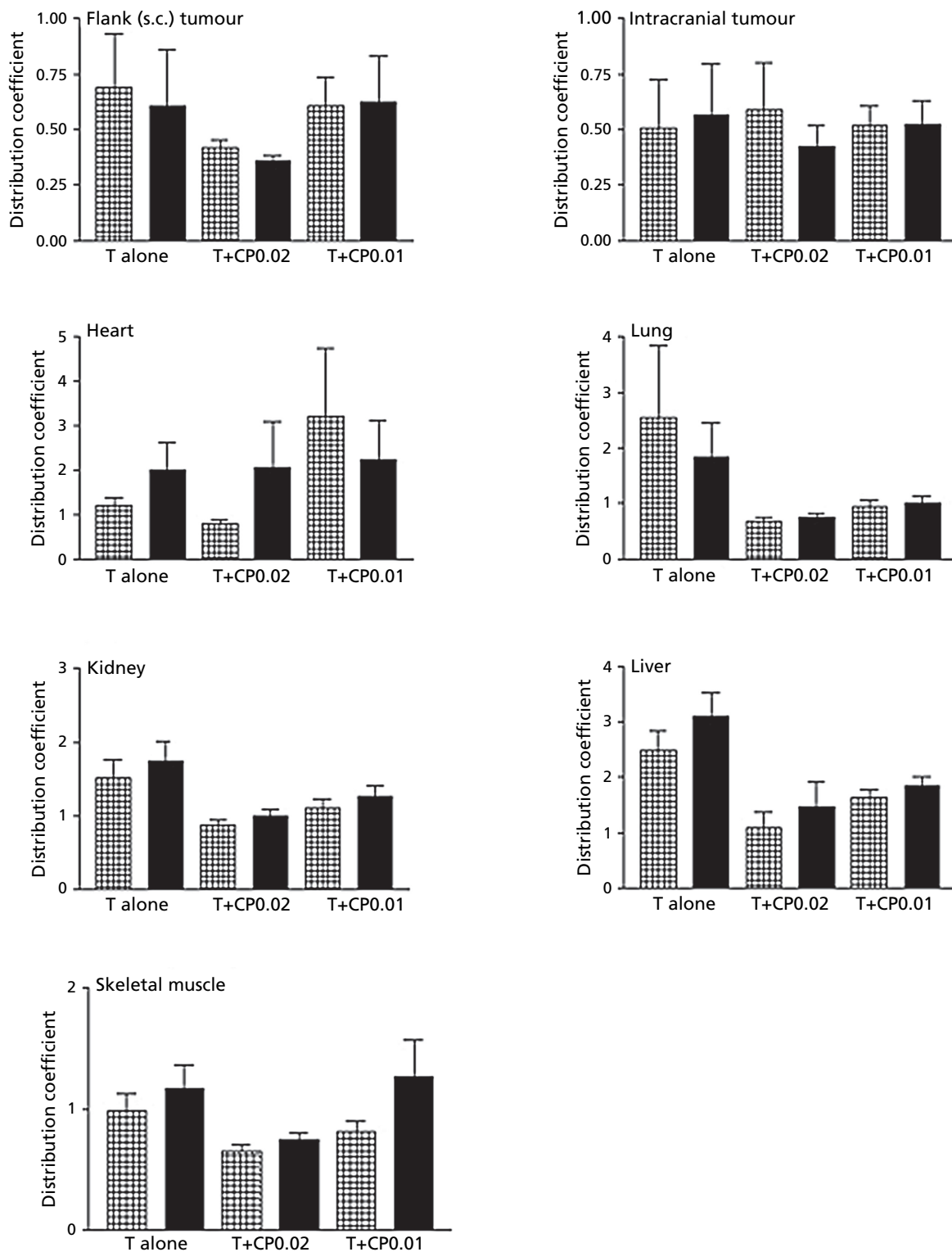
Figure 4 Mean (± s.e.m.) distribution coefficients of *R*- and *S*-thalidomide (respectively, checked and solid bars) in subcutaneous flank tumours, intracranial tumours, heart, lung, kidney, liver and skeletal muscle when thalidomide was administered to rats alone (T alone), and with cisplatin 0.01 mg kg^{-1} (T+CP0.01) or cisplatin 0.02 mg kg^{-1} (T+CP0.02). Data correspond to those shown in Figure 3.
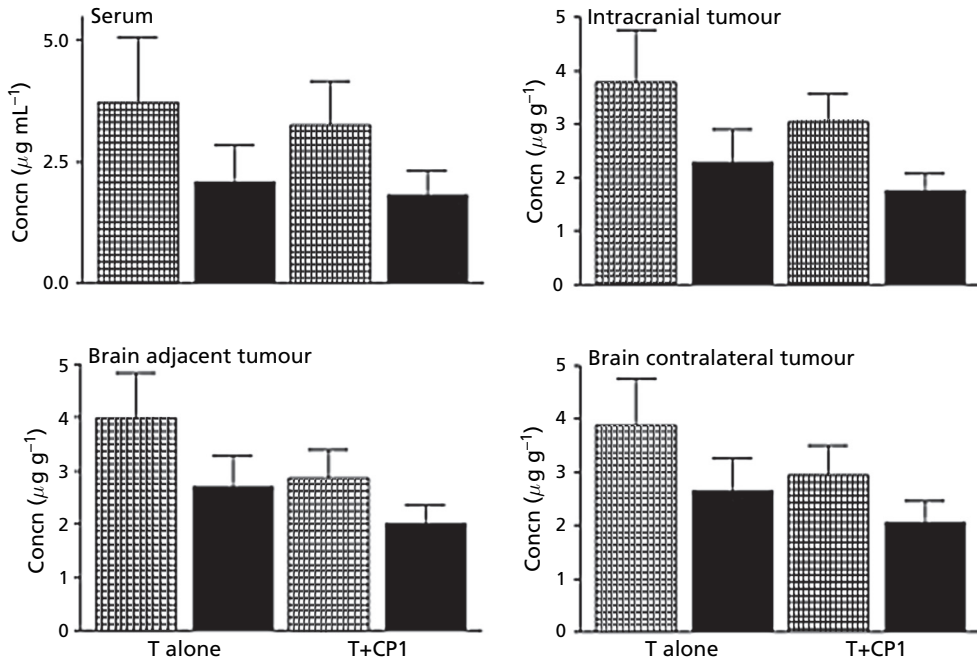
Figure 5 Mean (± s.e.m.) concentrations of *R*- and *S*-thalidomide (respectively, checked and solid bars) found in serum, intracranial tumour and brain adjacent to intracranial tumour and contralateral to the intracranial tumour from rats treated with thalidomide (T alone) and with cisplatin 1 mg kg^{-1} (T+CP1).
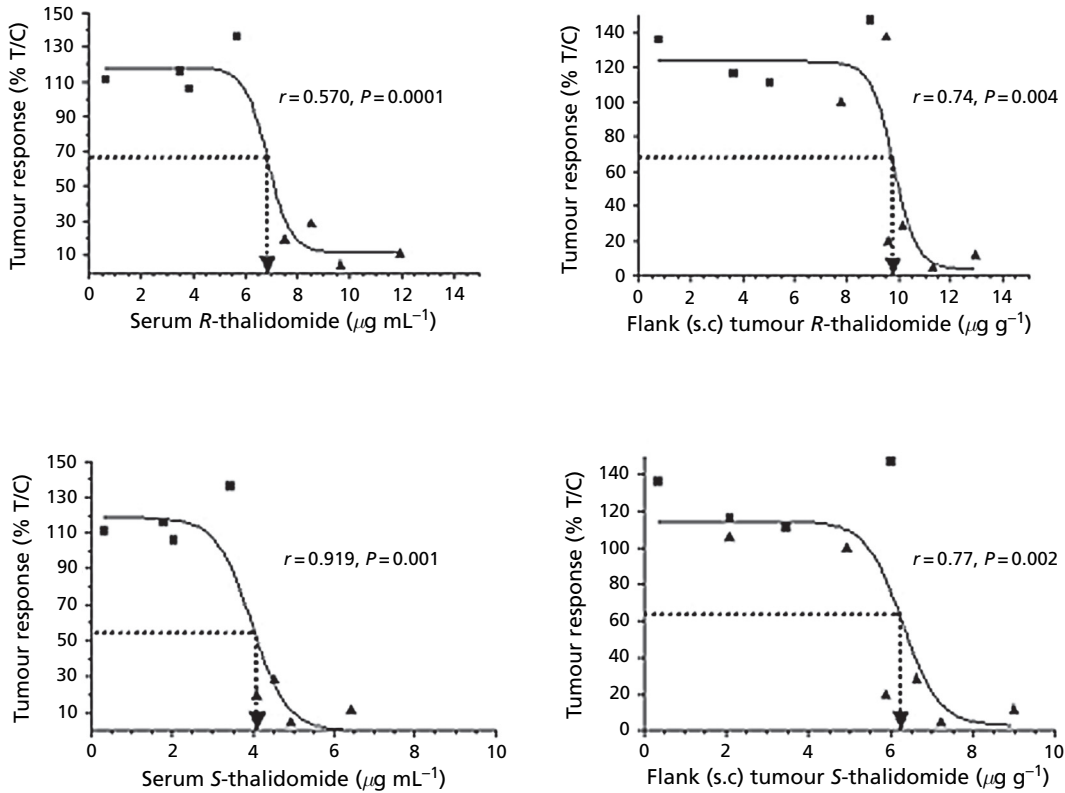
Figure 6 Scatterplots of combined anti-tumour response and serum and subcutaneous tumour concentrations of *R*- and *S*-thalidomide found in rats treated with thalidomide (square symbols), thalidomide + cisplatin 0.01 mg kg^{-1} and thalidomide + cisplatin 0.02 mg kg^{-1} (triangle symbols). Each point is derived from one subject. From the combined data (i.e. both enantiomers concurrently), and in the absence and presence of cisplatin, when fitted with 4-point sigmoidal curves these respective estimated EC50 values for *S*-thalidomide (approximately 4.0 and 6.2 μg g^{-1}) were smaller than those of *R*-thalidomide (approximately 6.8 and 9.9 μg g^{-1}).

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