In vivo studies of a platinum(II) metallointercalator†

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An *in vivo* study for determining the toxicity and efficacy of [Pt(*S*,*S*-dach)(phen)Cl_2·1.5H_2O·0.5HCl (PHENSS) in female Specific Pathogen Free (SPF) Swiss nude mice bearing PC3 tumour xenografts revealed PHENSS to be non-toxic and effective in decreasing tumour growth.

Many platinum(II) complexes have been investigated as anticancer drugs over the past forty years.[1–10] A large proportion of these drugs were derived from the clinically successful anticancer agent cisplatin (*cis*-diamminedichloroplatinum(II), CDDP) (Fig. 1). Cisplatin's biological activity results from its irreversible coordination to N7 atoms of adenine and guanine bases in DNA.[11] Numerous attempts have been made to develop platinum drugs (and to a lesser degree, non-platinum drugs) that overcome the problems associated with current platinum-based cancer treatments. New drugs should be selectively toxic against cancer cells, show activity against tumours that are resistant to cisplatin[12] and/or possess properties superior to existing clinically used anticancer drugs (*e.g.* reduced dose-related side effects, lower toxicity).[7] The initial therapeutic assessment of anticancer agents is commonly conducted through *in vitro* cytotoxicity experiments, using a variety of human and animal tumour cell lines,[13–18] followed by *in vivo* studies employing animal models to evaluate the toxicity and efficacy of potential drugs.[19]

We recently reported the therapeutic potential of four chiral platinum(II) metallointercalators, based on *in vitro* cytotoxicity experiments in numerous cancer cell lines.[18] Metallointercalators bind to DNA *via* intercalation, a reversible insertion of the drug's planar surface area between adjacent DNA base pairs, which is stabilised by π–π stacking and dipole–dipole interactions.[20–22] This interaction leads to a distortion in the topology of the DNA which causes a blockade of functions at the biochemical level.[20,23,24] *Trans*-(1*S*,2*S*)-1,2-diaminocyclohexane-1,10-phenanthrolineplatinum(II) perchlorate, [Pt(*S*,*S*-dach)(phen)]$(ClO_4)_2$,[15,18] exhibited greater cytotoxicity compared with its *R*,*R*-enantiomer, methylated analogues and CDDP. The cytotoxicity of the water-soluble analogue, [Pt(*S*,*S*-dach)(phen)]Cl_2·1.5H_2O·0.5HCl (PHENSS; the form of the complex used in the present study) (Fig. 1), and CDDP in three tumour cell lines are summarised in Table 1. PHENSS exhibits higher cytotoxicity than CDDP, and is greater than 10 times more biologically active against both the L1210/CDDP and PC3 cell lines.

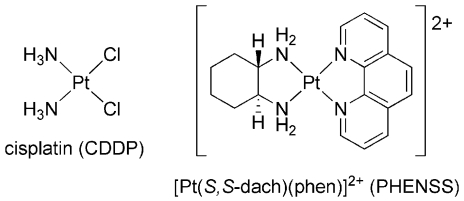

Fig. 1 Cisplatin (CDDP) and $[Pt(S,S\text{-dach})(phen)]^{2+}$ (PHENSS).

In the present study, preliminary *in vivo* assays were conducted to determine the maximum tolerated dose (MTD) and optimal dosage regime for PHENSS and to evaluate the toxicities and efficacies of the drugs in specific pathogen free (SPF) mice. A good correlation between pre-clinical studies and clinical toxicology data in human trials exist for a number of platinum anticancer compounds.[25] Three experiments were conducted using different mouse models and CDDP as a postive control: (i) an acute intraperitoneal toxicity sighting study; (ii) a repeated-dose intraperitoneal toxicity sighting study; and (iii) a 20 day repeated-dose intraperitoneal efficacy and toxicity study. The acute intraperitoneal toxicity‡ sighting study (Study 1) was used to examine toxicity and thus determine the highest NOAEL (no observed adverse effects level) for PHENSS and CDDP in naive mice. The repeated-dose intraperitoneal toxicity§ sighting study (Study 2) was used to determine a suitable dose regime in nude mice from which a single dosage level of PHENSS and CDDP would be selected for the main efficacy study (Study 3). The 20 day repeated-dose

Table 1 *In vitro* cytotoxicities (IC_{50}) of platinum(II) complexes in tumour cell lines

	$IC_{50} \pm SD^b$ (μM)	
Cell line[a]	PHENSS	CDDP
L1210	0.19 ± 0.01	0.50
L1210/CDDP	0.20 ± 0.04	6.90
PC3	0.52 ± 0.12	5.60

[a] L1210 = murine leukaemia; L1210/CDDP = cisplatin resistant murine leukaemia; PC3 = human prostate carcinoma. [b] Values with standard deviations (SD) are averages of at least three independent determinations; values without SD are averages of two determinations.

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intraperitoneal efficacy and toxicity study (Study 3) was used to further investigate the toxicity of PHENSS, and to determine whether the drug was effective in preventing the growth of tumours induced by xenograft of the tumour cell line PC3 into outbred nude mice. Nude mice have no thymus (thus making them immune-deficient) and were used in Studies 2 and 3 to maximise the induced tumourigenic effect. *In vivo* experiments were conducted by ICP Firefly Pty Ltd., an Animal Research Establishment accredited with NSW Agriculture (Ref. No. AW96/042). All work undertaken by ICP Firefly Pty Ltd. complies with the NSW Government Legislation (The Animal Research Act 1985 and Regulation 1995) and was approved by the Animal Ethics Committee (#E0819) constituted by the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 1997 (National Health and Medical Research Council).

Study 1 revealed no clinical signs of toxicity in mice groups treated with either 2, 4, 8, 12 or 16 mg kg^{-1} of PHENSS, or with 2, 4 or 8 mg kg^{-1} of CDDP. A rough coat¶ was briefly observed throughout the study for all mice in the 32 mg kg^{-1} PHENSS and 12 mg kg^{-1} CDDP treated groups. Increases in body weight (Table S1)† were observed for most groups throughout the study (no dose-dependency observed), though a notable decrease in body weight was recorded on days 4 and 8 (ave. 21.5 g) for the 12 mg kg^{-1} CDDP group, compared with 23.5 g and 24.2 g on days 1 and 15, respectively. No gross abnormalities were observed in major organs (*i.e.*, liver, kidneys, adrenals, gonads and spleen) of any animal at necropsy. The MTDs were determined to be between 16 to 32 mg kg^{-1} for PHENSS and between 8 to 12 mg kg^{-1} for CDDP (literature MTDs for CDDP: 4 mg kg^{-1} in CD1 nude mice;[26] 12 mg kg^{-1} in BDF1 mice).[27] Based on the experimental findings of Study 1, the NOAELs of 4, 8, 12 and 16 mg kg^{-1} for PHENSS and 4 and 8 mg kg^{-1} for CDDP were selected for use in the subsequent study.

No clinical signs of toxicity and no premature deaths were observed during the 20 days of Study 2. Over this period, increases in body weight (Table S2)† were observed across all groups, with the exception of Group 10 (8 mg kg^{-1} of CDDP every six days). During the first 15 days of Study 2, Group 10 had a lower body weight gain (compared with other groups), with a 4% decrease in body weight between days 15 to 21 (Table S2).† One animal in Group 7 (12 mg kg^{-1} of PHENSS every four days) was observed to have an enlarged spleen at necropsy; though this animal was also found to have only one adrenal and one kidney (not related to treatment with PHENSS). No other gross pathology was found in the animals of any other group. The results of Study 2 showed PHENSS to have no toxic effects on nude mice at the doses and regimens tested; while, CDDP at 8 mg kg^{-1} caused a decrease in body weight. Thus, the suitable single dosage regimes selected for Study 3 were 16 mg kg^{-1} of PHENSS (given every two days) and 8 mg kg^{-1} of CDDP (given every six days).

Study 3 was used to determine the effectiveness of PHENSS in preventing the growth of PC3 tumours (induced by xenograft) in nude mice. Three groups of mice were injected with the PC3 tumour cell line on day 1 and treated with the test compounds according to the dosage regimes established in Study 2. The intended duration of this study was 23 days or until the first mortalities. Mice in Groups 1 and 2 were treated with PHENSS or CDDP commencing day 3, and Group 3 with sterile saline (negative control) from day 1. By day 20, three of the mice in the CDDP treated group (Group 2) had died. The study was terminated due to these mortalities and the remaining mice were sacrificed on day 20 (no premature deaths were recorded in the PHENSS or negative control group).

Body weight gains for the PHENSS treated mice (Group 1) and the negative control animals (Group 3) were comparable throughout the 20 day study period (Fig. 2). On day 15 the CDDP treated mice (Group 2) were observed to be leaner, compared to the mice in the PHENSS and control groups. By day 20, significant reductions in body weight (mean weight loss = 28%) were observed for the animals in Group 2 (including the three mice that died) compared with the control mice (Fig. 2). No gross abnormalities were detected in the major organs of any animal at necropsy. There was, however, a trend for higher liver weights in the PHENSS treated mice (Group 1) compared with the control (Group 3). The cause of this increase is unknown, as no toxicity was observed in these animals; histopathology on the livers (to be performed in the near future) may provide additional insight. The liver weights and spleens of the CDDP treated animals (Group 2) were comparatively smaller than those in Groups 1 and 3.

Palpable tumours were first observed on the mice in all groups (Groups 1, 2 and 3) on day 5 of Study 3. Marginal differences in tumour volumes were observed between the groups treated with PHENSS and CDDP and the control group over the study period (Fig. 3). However, on day 19 a lower mean tumour volume of 914.1 ± 333.5 mm^3 was observed for the PHENSS treated mice (Group 1) compared with the control Group 3 (1567.5 ± 642.7 mm^3, $P = 0.061$). This observation is considered to be biologically significant, as the mean tumour volume was decreased by approximately 40% following treatment with PHENSS. The mean tumour volumes of the CDDP treated mice (Group 2) were also significantly decreased when compared with the control animals on day 19 of the study, at 737.9 ± 512.4 mm^3 ($P = 0.034$) (Fig. 3), though three out of six mice in Group 3 died the following day (day 20).

Differences in mean tumour weights (measured at necropsy) between the three groups of mice in Study 3 (Table 2) were not

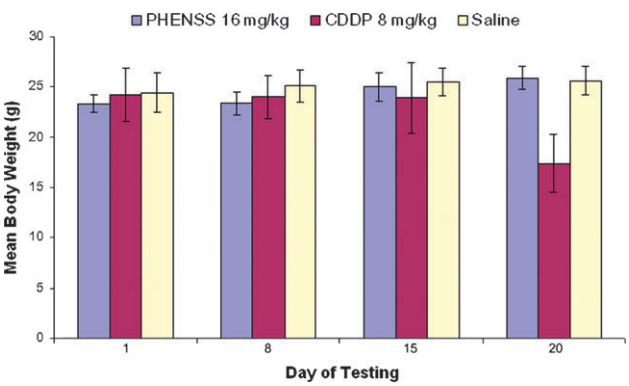

Fig. 2 Mean body weights of SPF Swiss nude mice bearing PC3 tumours (Study 3) treated with PHENSS (Group 1), CDDP (Group 2) and saline (Group 3) (no. mice per group = 6).

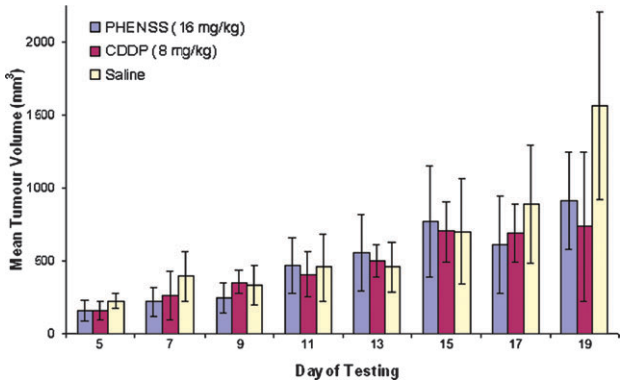

Fig. 3 Mean PC3 tumour volumes in Swiss SPF nude mice (Study 3) treated with PHENSS (Group 1), CDDP (Group 2) and saline (Group 3) (no. mice per group = 6).

Table 2 Mean PC3 tumour weights in SPF Swiss nude mice (Study 3)

Group	Test compound	No. of mice	Mean tumour weight (± SD) (mg)
1	PHENSS (16 mg kg^{-1})	6	29 ± 14
2	CDDP (8 mg kg^{-1})	3 (3 died)	26 ± 7.0
3	Saline	6	44 ± 24

statistically significant due to wide variations in the tumour volumes calculated (Fig. 3), although lower mean tumour weights were observed for both the PHENSS and CDDP treated groups when compared to the saline control Group 3 (35–40% decrease was observed). This trend in tumour weight may also have some degree of biological significance.

Under the conditions of Study 3, PHENSS did not produce obvious signs of toxicity in female SPF nude mice bearing the PC3 tumour cell xenografts. Although treatment with PHENSS did not significantly retard tumour growth over the 20 day study period, a trend was observed in Group 1 with mice having lower tumour volumes and tumour weights than the saline treated animals (Group 3). Study 3 was terminated on day 20 due to the deaths of three mice in the CDDP treated group (Group 2).

The results of Study 3 suggest that the efficacy of PHENSS should be further explored to determine if this compound can produce a statistically significant antitumour effect (*i.e.*, $P < 0.05$). Small sample sizes used in Study 3 ($n = 6$) are likely to account for large deviations in the measurements of body weights, tumour weights and volumes, *etc.*, thus having a large impact on the variance of the data. An additional study over a longer observation period (*e.g.* 50 days, or until a significant loss in body weight is observed) with increased sample sizes and using a lower treatment dose of CDDP (<8 mg kg^{-1}) may provide a more accurate evaluation of the efficacy of PHENSS. Further *in vivo* evaluation of PHENSS has been scheduled for the near future.

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Notes and references

‡ Acute intraperitoneal toxicity is an adverse non-specific effect that occurs in an animal within a short time after being injected with a single dose of the drug (these effects are continually observed over a defined period).
§ Repeated intraperitoneal toxicity is an undesirable non-specific effect that is observed in an animal within a short time frame after being injected with several repeated doses of the drug over a defined period (usually one to two weeks).
¶ A rough coat (generally considered a clinical sign of toxicity) indicates that these mice were unwell and may have died within a few days after this observation.

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