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PAPER

A new platinum-complex showing easy preparation, promising anti-tumor activity, and better efficacy and distribution properties than oxaliplatin

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Current clinically used chemotherapeutic platinum drugs can trigger severe toxic effects. To develop a model system for the evaluation of the therapeutic efficacy and the toxic effects of new platinum agents, we have synthesized a new compound *N*-[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxylhex-1-yl]-L-hydroxyproline dichloroplatinum(II) (PHDP), compared its *in vitro* anti-proliferation activity, *in vivo* anti-tumor activity and safety to those of oxaliplatin, and correlated all these biological actions with the platinum occurring in the spleen, kidney, heart, brain, blood, tumor tissue, urine and faeces of the treated mice. We explored the atomic absorption based determinations of the platinum which occurred in the spleen, kidney, heart, brain, blood, tumor tissue, urine and faeces and constitute a model system that can be generally used in the investigation of the novel platinum agents.

1. Introduction

The discovery of *cis*-diamminedicholoroplatinum(II) (cisplatin) over 50 years ago was a significant milestone in the clinical chemotherapies of cancer. Since then, cisplatin and its derivatives, *cis*-diamminecyclobutane dicarboxylatoplatinum(II) (carboplatin) and (*trans*-*R*,*R*-cyclohexane-1,2-diamine)-oxalatoplatinum(II) (oxaliplatin), have been widely used for treating various solid tumors.[1–5] As first line chemotherapeutic agents, cisplatin is now used to treat testicular, ovarian, head and neck, cervical and bladder cancers;[6,7] carboplatin is used to treat small-cell and non-small cell lung cancers;[8] and oxaliplatin is used to treat colorectal cancer and metastatic colorectal cancer.[9–11] However, the intrinsic resistance,[12] *in vivo* toxicities and severe side effects such as neurotoxicity, nephrotoxicity, ototoxicity, myelosuppression, nausea and vomiting, limit the clinical utility of these platinum(II) complexes.[13,14] These toxicities and side reactions are usually attributed to the lack of selective delivery of platinum(II) to the tumor tissue,[15] and various strategies have been developed in attempt to combat this problem.[16–24]

In general, nephrotoxicity results from the accumulation of platinum in the kidneys, the accumulation of platinum(II) in the brain is the cause of neurotoxicity and ototoxicity, while the accumulation of platinum in the blood causes myelosuppression. Based on these notions, an ideal model system capable of estimating the anti-cancer activity, the toxicity and the side effects of platinum agents should be characterized by the accumulation of platinum(II) in the organs, blood, urine and tumors.

In the co-administration of cisplatin and glucosyl dithiocarbamate-amino acids, a complex consisting of Pt(II) and sugar-amino acid derivatives was found and correlated with the benefits.[25] We consider this kind of Pt(II) complex an active metabolite and could be a lead for designing novel chemotherapeutic agents. Here, this complex was used as a sugar containing platinum framework, simplified glucosyldithiocarbamate-amino acid to pentahydroxyhexylamino acid, and *N*-[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxylhex-1-yl]-L-hydroxyproline dichloroplatinum(II) (PHDP) was provided as a novel anti-cancer agent. Its *in vitro* anti-proliferation and *in vivo* anti-tumor activities, as well as the therapy caused accumulation of platinum in the organs, blood, urine and tumor of the treated mice were compared to those from the therapy of oxaliplatin, an excellent representative of platinum(II) complexes.

2. Results

2.1 Preparing PHDP *via* a two-step-procedure

The synthesis of *N*-[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxylhex-1-yl]-L-hydroxyproline dichloroplatinum (PHDP) was characterized by a two-step-procedure. In the first step *N*-[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxylhex-1-yl]-L-hydroxyproline was prepared (in 22% yield) *via* a one-pot-three-step synthesis consisting of (a) the condensation of glucose and L-hydroxyproline, (b) the reduction of the condensation product and (c) acidifying the sodium carboxylate. In the second step, PHDP was prepared (in 81% yield) *via* one-pot-two-step

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synthesis consisting of (a) the addition of *N*-[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxylhex-1-yl]-L-hydroxyproline and $K_2[PtCl_4]$, and (b) neutralizing the sodium salt of PHDP and the excessive sodium hydroxide. The structure of PHDP was confirmed with IR, ^{1}H NMR and elemental analysis.

2.2 *In vitro* anti-proliferation activities of PHDP and oxaliplatin

The *in vitro* anti-proliferation activities of PHDP and oxaliplatin were evaluated with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assays according to the standard procedure and expressed by their IC_{50} of inhibiting the growth of H1299, HL_{60}, HepG2, HeLa and MES-SA cells. The IC_{50} values are shown in Fig. 1. For the growth inhibition of the five cells, the IC_{50} values of PHDP and oxalipatin range from 3.60 to 11.26 μM and from 4.90 to 13.65 μM, respectively. These mean that both PHDP and oxalipatin exhibit effective *in vitro* anti-proliferation action, but the *in vitro* anti-proliferation activities of oxalipatin are significantly lower than that of PHDP.

2.3 *In vivo* anti-tumor activities of PHDP and oxaliplatin

The *in vivo* anti-tumor activities of PHDP and oxaliplatin were evaluated by using S180 mice, the tumor weights are shown in Fig. 2. After treating for ten consecutive days the tumor weight of the mice receiving 16.7 μmol kg^{-1} of PHDP is 414.8 mg, which is significantly lower than that of the mice receiving NS (normal saline) (1316.2 mg). This means that 16.7 μmol kg^{-1} of PHDP exhibits *in vivo* anti-tumor action. After treating for ten consecutive days the tumor weight of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin is 716.1 mg, which is also significantly lower than that of the mice receiving NS. This means that 16.7 μmol kg^{-1} of oxaliplatin exhibits *in vivo* anti-tumor action. However, the tumor weight of the mice receiving 16.7 μmol kg^{-1} of PHDP is significantly lower than that of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin. Thus, at this dose the *in vivo* anti-tumor efficacy of PHDP is significantly higher than that of oxaliplatin.

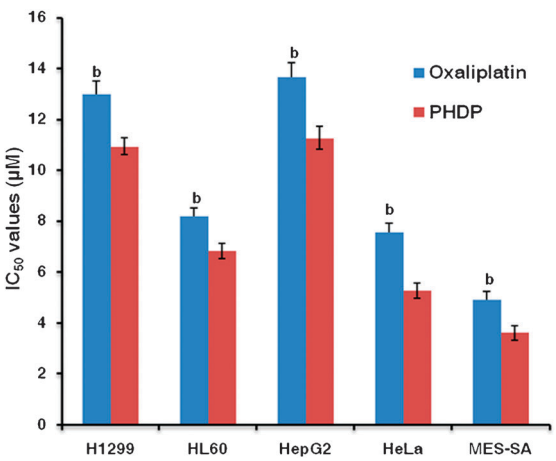

Fig. 1 IC_{50} values of PHDP and oxalipatin inhibiting the growth of the cancer cells. IC_{50} is expressed by $\bar{x} \pm$ SD μM, $n = 6$; (b) Compared to PHDP $p < 0.01$.

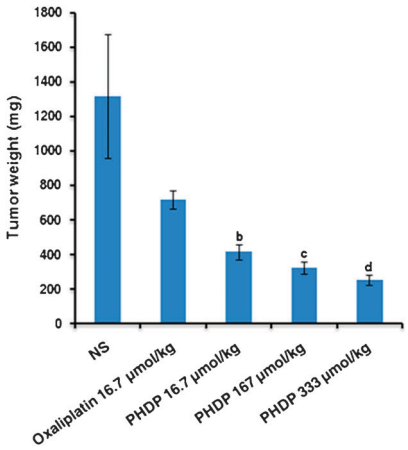

Fig. 2 Tumor weights of mice receiving PHDP and oxaliplatin. Tumor weight is represented by $\bar{x} \pm$ SD mg, NS (normal saline) = vehicle, $n = 10$; (b) Compared to NS, 16.7 μmol kg^{-1} of oxaliplatin, as well as 167 and 333 μmol kg^{-1} of PHDP $p < 0.01$; (c) Compared to NS, 16.7 μmol kg^{-1} of oxaliplatin and 333 μmol kg^{-1} of PHDP $p < 0.01$; (d) Compared to NS and 16.7 μmol kg^{-1} of oxaliplatin $p < 0.01$.

2.4 Dose dependent action of PHDP

The clinical dosage of oxaliplatin is 16.7 μmol kg^{-1}, and here this dosage was used for PHDP as a normal dosage to perform the *in vivo* anti-tumor assays. To observe the effect of dose on the activity of PHDP, 16.7 μmol kg^{-1} was extended 10 fold (167 μmol kg^{-1}) and 20 fold (333 μmol kg^{-1}) and these results are also shown in Fig. 2. After treating for ten consecutive days the tumor weights of the mice receiving 167 and 333 μmol kg^{-1} of PHDP are 325.3 mg and 252.8 mg, respectively. The statistical analysis gave it an activity order of 333 μmol kg^{-1} > 167 μmol kg^{-1} > 16.7 μmol kg^{-1}. This means that PHDP inhibits dose-dependently the growth of the tumor *in vivo*.

2.5 Organ weights of the mice receiving PHDP and oxaplatin

One of the visible toxic effects of platinum in the organs could lead the organs to atrophy. To evaluate the organ toxicity, the organ weights of the mice receiving PHDP and oxaliplatin were measured according to the mentioned procedure and are shown in Fig. 3. After treating for ten consecutive days, the weights of the spleen, kidney, heart and brain of the mice receiving 16.7 and 167 μmol kg^{-1} of PHDP are equal to that of the mice receiving NS. This means that 16.7 and 167 μmol kg^{-1} of PHDP do not lead to atrophy of the spleen, kidney, heart and brain of the treated mice. The weights of the spleen, kidney, heart and brain of the mice receiving 333 μmol kg^{-1} of PHDP are significantly lower than that of the mice receiving NS. This means that 333 μmol kg^{-1} of PHDP leads to atrophy of these organs. On the other hand, the weights of the spleen, kidney, heart and brain of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin are significantly lower than that of the mice receiving NS. This means that even 16.7 μmol kg^{-1} of oxaliplatin could lead to atrophy of these organs.

2.6 Survival of the mice receiving PHDP and oxaliplatin

The organ toxicity of the platinum therapy may ultimately affect the survival rate of the treated mice. To explore the

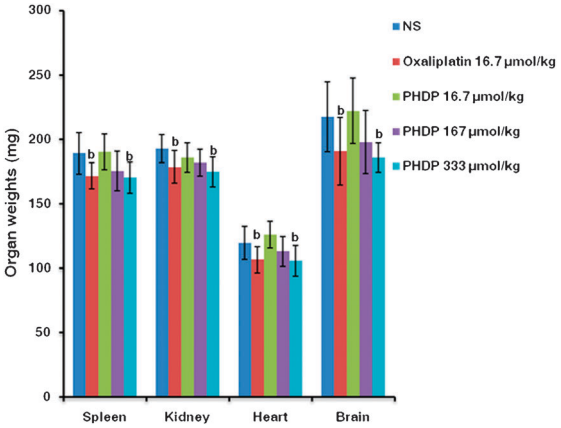

Fig. 3 Organ weights of the mice receiving PHDP and oxaliplatin. $n = 10$, NS (normal saline) = vehicle, organ weight is represented by $\bar{x} \pm$ SD mg; (b) Compared to NS $p < 0.05$.

ultimate effect of the organ toxicity, the survival rate of the mice receiving PHDP and oxaliplatin were tested and are shown in Fig. 4. At 16.7 and 167 μmol kg^{-1} doses, the survival rate of the 10 mice receiving PHDP is 100%. This means that at both doses PHDP exhibits sufficient safety profile. Besides, the survival rates of the 13 mice receiving 16.7 μmol kg^{-1} of oxaliplatin and the 13 mice receiving 333 μmol kg^{-1} of PHDP are the same, *i.e.*, 90% on the 8th day and 80% on the 10th day. This means that the safety of PHDP is 20 folds higher than that of oxaliplatin.

2.7 Body weights of the mice receiving PHDP and oxaplatin

For platinum therapy the toxicity may result in the general asthenia such as the body emaciation. To find the effect of the toxicity on the asthenia, the body weights of the mice receiving PHDP and oxaliplatin were measured. Fig. 5 indicates that after treating for ten consecutive days, the body weights of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin are equal to that of the mice receiving NS. This implies that at this dose, the toxicity of oxaliplatin does not affect the body weight of the treated mice. After treating for ten consecutive days, the body weights of the mice receiving 16.7, 167 and 333 μmol kg^{-1} of PHDP are also equal to that of the mice receiving NS. This means that even at doses of 333 μmol kg^{-1}, the toxicity of PHDP still does not affect the body weight of the treated mice.

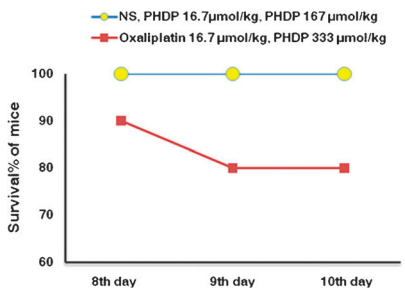

Fig. 4 Survival% of mice receiving PHDP and oxaliplatin. Survival is represented by mice %, NS (normal saline) = vehicle, $n = 10$, and the survival of 16.7 μmol kg^{-1} of cisplatin treated mice was 40%.[26]

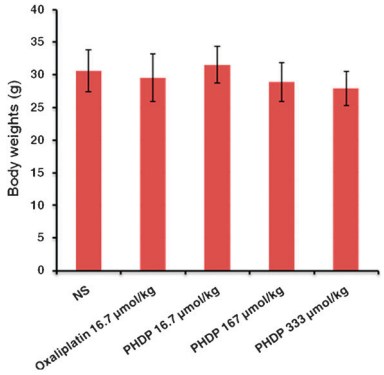

Fig. 5 Body weights of mice receiving PHDP and oxaliplatin. $n = 10$, NS (normal saline) = vehicle, body weight is represented by $\bar{x} \pm$ SD g.

2.8 Organ platinum of mice receiving PHDP and oxaliplatin

The organ damage of the platinum therapy may be generally attributed to the accumulation of the platinum in the organs of the treated mice. By atomic absorption based analysis, the platinum accumulated in the spleen, kidney, brain and heart of the mice receiving PHDP and oxaliplatin was measured.

Fig. 6A indicates that after treating for ten consecutive days, the platinum accumulated in the spleen of the mice receiving 16.7 μmol kg^{-1} and 167 μmol kg^{-1} of PHDP is significantly lower than that and equal to that of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin, respectively. This means that for mice, the damage to the spleen induced by PHDP therapy is 10 folds lower than that induced by oxaliplatin therapy. The platinum accumulated in the kidney of the mice receiving 333 μmol kg^{-1} of PHDP is equal to that of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin. This means that for mice, the damage to the kidney induced by PHDP therapy is 20 folds lower than that induced by oxaliplatin therapy.

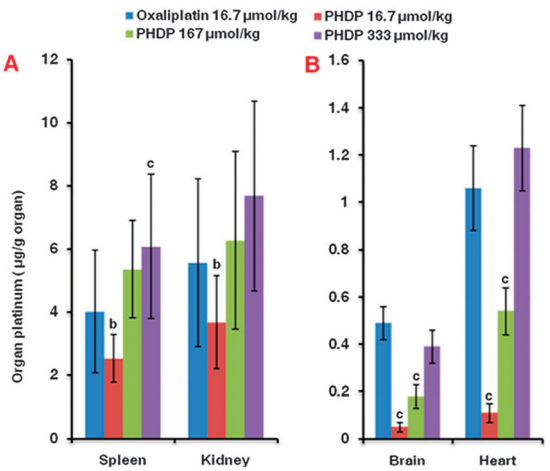

Fig. 6 Organ platinum of mice receiving PHDP and oxaliplatin. Organ platinum is represented by $\bar{x} \pm$ SD μg of platinum per g of organ, $n = 10$ and the platinum in the spleen, kidney, brain and heart of 16.7 μmol kg^{-1} of cisplatin treated mice were 6.05 ± 1.35, 12.41 ± 2.67, 0.70 ± 0.22 and 1.58 ± 0.59 μg of platinum per g of tissue, respectively.[26] (A) For spleen and kidney, compared to oxaliplatin (b) $p < 0.01$ and (c) $p < 0.05$. (B) For brain and heart, compared to oxaliplatin (c) $p < 0.01$.

Fig. 6B indicates that after treating for ten consecutive days the accumulation of the platinum in the brain and heart of the mice receiving 16.7 and 167 μmol kg^{-1} of PHDP is significantly lower than that of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin. This means that for mice, even at the dose of 167 μmol kg^{-1}, the brain and heart damage induced by PHDP therapy are still significantly lower than that induced by oxaliplatin therapy. Besides, the platinum accumulated in the brain and heart of the mice which received 333 μmol kg^{-1} of PHDP is equal to that of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin. This means that for mice the damage of brain and heart induced by PHDP therapy is 20 folds lower than that induced by oxaliplatin therapy.

2.9 Blood platinum of mice receiving PHDP and oxaliplatin

Blood toxicity of platinum therapy may generally correlate with the concentration of blood platinum. By analysis based on atomic absorption, the accumulation of the platinum in the blood of the mice which received PHDP and oxaliplatin were measured. Fig. 7 indicates that after treating for ten consecutive days the accumulation of the platinum in the blood of the mice receiving 16.7 and 167 μmol kg^{-1} of PHDP is significantly lower than that of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin. This means that for mice, the blood toxicity of both 16.7 and 167 μmol kg^{-1} of PHDP therapies is significantly lower than that of 16.7 μmol kg^{-1} of oxaliplatin therapy. Besides, the accumulation of the platinum in the blood of the mice receiving 333 μmol kg^{-1} of PHDP is equal to that in the blood of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin. This means that for mice the blood toxicity of PHDP therapy is 20 folds lower than that of oxaliplatin therapy.

2.10 Urine and fecal platinum of mice receiving PHDP and oxaliplatin

To evaluate the ability of PHDP to be eliminated from the organs, the urine and faecal platinum of the mice receiving PHDP and oxaliplatin were tested according to the mentioned procedure.

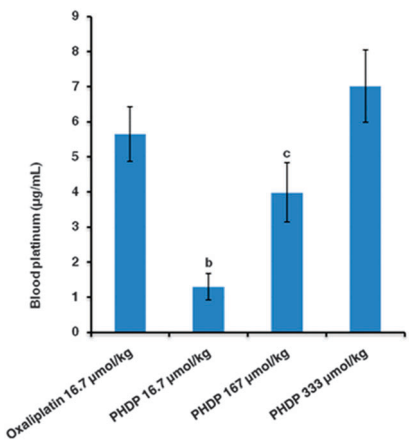

Fig. 7 Blood platinum of mice receiving PHDP and oxaliplatin. Blood platinum is represented by $\bar{x} \pm$ SD μg of platinum per mL of blood, $n = 10$, and the blood platinum of 16.7 μmol kg^{-1} of cisplatin treated mice was 6.19 ± 1.11 μg of platinum per mL of blood;[26] (b) Compared to oxaliplatin $p < 0.01$; (c) Compared to oxaliplatin $p < 0.05$.

The results are shown in Fig. 8. The urine and faecal platinum of the mice receiving 16.7, 167 and 333 μmol kg^{-1} of PHDP are dose dependently increased. This means that PHDP is dependently eliminated from the organs. Besides, the urine and faecal platinum of the mice receiving 16.7, 167 and 333 μmol kg^{-1} of PHDP is significantly higher than that of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin. This suggests that PHDP is more easily eliminated from the organs of the treated mice than oxaliplatin.

2.11 Urine quantity of mice receiving PHDP and oxaliplatin

Increasing urination is an important way for the treated mice to protect them from the damage of platinum accumulation. To define the self-protection of the mice receiving PHDP, the urine quantities of the treated mice were measured and the data are shown in Fig. 9. As seen, the urine quantity of mice receiving 16.7 μmol kg^{-1} of PHDP is significantly higher than that of the mice receiving NS and 16.7 μmol kg^{-1} of oxaliplatin. This means that at this dose, PHDP increases the urination of mice and consequently limits the damage of platinum accumulation. Besides, the urine quantities of mice receiving 16.7, 167 and 333 μmol kg^{-1} of PHDP are 2.56 mL, 2.74 mL and 2.88 mL, respectively. The statistical analysis defines a urine quantity order of 333 μmol kg^{-1} > 167 μmol kg^{-1} > 16.7 μmol kg^{-1}, and means that PHDP dose-dependently benefits mice to urinate.

2.12 Tumor platinum of mice receiving PHDP and oxaliplatin

Keeping a high accumulation of the platinum in the tumor tissue of the treated mice is a critical indicator of the platinum therapy. To reveal the accumulation of the platinum in the tumor tissue of the mice receiving PHDP and oxaliplatin, atomic absorption based analysis was performed. As seen in Fig. 10, after treating for ten consecutive days the accumulation of the platinum in the tumor tissue of the mice receiving 16.7 μmol kg^{-1} of PHDP is significantly higher than that of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin. This means that compared to oxaliplatin, PHDP induces a higher accumulation of the platinum in the tumor issue of the treated mice.

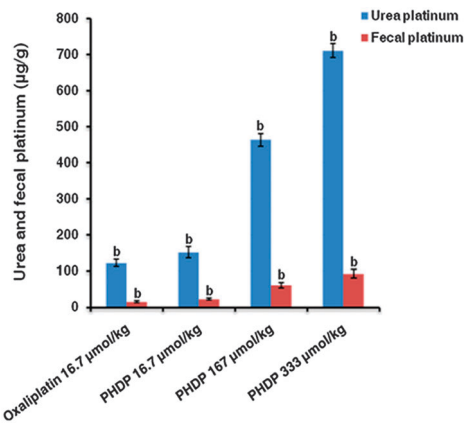

Fig. 8 Urine and fecal platinum of mice receiving PHDP and oxaliplatin. Urine and faecal platinum is represented by $\bar{x} \pm$ SD μg of platinum per g of urine and fecal; (b) Compared to oxaliplatin $p < 0.01$.

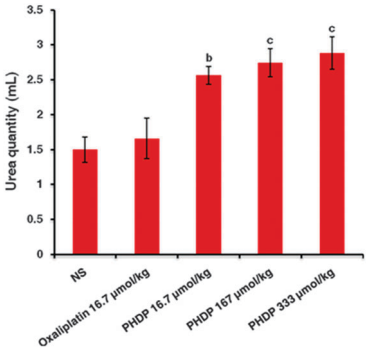

Fig. 9 Urine quantity of mice receiving PHDP and oxaliplatin. Urine quantity is represented by $\bar{x} \pm$ SD mL, $n = 10$; (b) Compared to 16.7 μmol kg^{-1} of oxaliplatin and 333 μmol kg^{-1} of PHDP $p < 0.01$, to 167 μmol kg^{-1} of PHDP $p < 0.05$; (c) Compared to 16.7 μmol kg^{-1} of oxaliplatin $p < 0.01$, to 167 μmol kg^{-1} of PHDP $p < 0.05$.

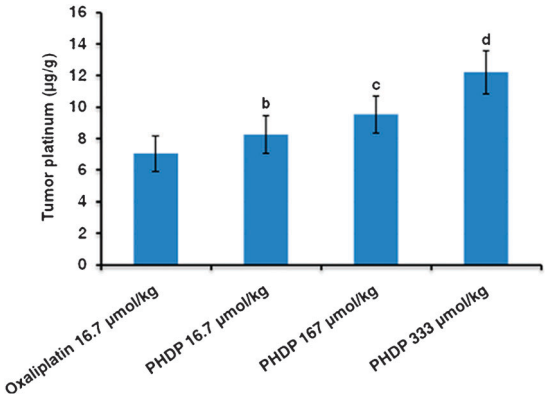

Fig. 10 Tumor platinum of mice receiving PHDP and oxaliplatin. $n = 10$, tumor platinum is represented by $\bar{x} \pm$ SD μg of platinum per g of tumor, and the platinum in the tumor issue of 16.7 μmol kg^{-1} of cisplatin treated mice was 5.29 ± 1.62 μg of platinum per g of tumor;[26] (b) Compared to oxaliplatin $p < 0.05$; (c) Compared to oxaliplatin $p < 0.01$, to 16.7 μmol kg^{-1} of PHDP $p < 0.01$; (d) Compared to oxaliplatin, as well as 16.7 and 167 μmol kg^{-1} of PHDP $p < 0.01$.

3. Discussion

3.1 Anti-tumor activity and tumor platinum

On the *in vitro* MTT model, PHDP and oxaliplatin effectively inhibit the proliferation of H1299, HL_{60}, HepG2, HeLa and MES-SA cells, and the IC_{50} values of the former are significantly lower than that of the latter. These observations imply that PHDP has higher anti-tumor efficacy than oxaliplatin *in vitro*. It was reported that compared to the neutrally charged agent, the *in vitro* uptake of a slightly negative charged agent towards tumor cells was higher.[27–29] The structure indicates that oxaliplatin and PHDP have an inherently neutral charge and slightly negative charge, respectively. This electrical difference between oxaliplatin and PHDP could be responsible for the difference between their *in vitro* anti-tumor activities.

On the *in vivo* S180 mice model, 16.7 μmol kg^{-1} of PHDP and 16.7 μmol kg^{-1} of oxaliplatin effectively inhibit the growth of the tumor, and the tumor weight of the mice receiving the former is significantly lower than that of the mice receiving the latter, therefore PHDP has higher anti-tumor efficacy than oxaliplatin *in vivo*. It is generally conceived that a high distribution of the platinum in the tumor tissue is essential for an effective therapy of platinum agent. The atomic absorption based analysis indicates that the accumulation of the platinum in the tumor issue of the mice receiving 16.7 μmol kg^{-1} of PHDP is significantly higher than that of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin. This observation implies that PHDP may induce higher accumulation of the platinum in the tumor tissue than oxaliplatin. It was also reported that compared to the neutrally charged agent, the *in vitro* uptake of a slightly negative charged agent towards tumor tissue is higher.[27–29] According to this, the distinct *in vitro* anti-tumor activities of the inherently neutrally charged oxaliplatin and the negatively charged PHDP are understandable.

3.2 Organ weights and organ platinum

One of the visible toxic reactions of platinum agents in the organs could lead to atrophy of the organs and consequently decreases the weight of the organs. The organ weight assay indicates that after treating for ten consecutive days, the weights of the spleen, kidney, heart and brain of the mice receiving 16.7 μmol kg^{-1} of PHDP are equal to that of the mice receiving NS. While the weights of the spleen, kidney, heart and brain of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin are significantly lower than that of the mice receiving NS. These mean that at this dose the visible toxic reaction of PHDP therapy is significantly lower than that of oxaliplatin therapy.

It is generally conceived that a low accumulation of the platinum in the spleen, kidney, heart and brain of the mice is essential for a platinum agent to exhibit low organ toxicity. The atomic absorption based analysis indicates that after treating for ten consecutive days the accumulation of the platinum in the spleen, kidney, heart and brain of the mice receiving 16.7 μmol kg^{-1} of PHDP is 1.7, 1.5, 9.0 and 9.6 folds lower than that of the platinum in the spleen, kidney, heart and brain of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin, respectively. These imply that compared to oxaliplatin, PHDP induces lower accumulation of the platinum in the spleen, kidney, heart and brain.

It was reported that to offer biological action the agent may initially be adsorbed to the cell surface with electrostatic force. Compared to tumor tissue (extracellular pH ~6.5), the extracellular pH of normal tissue is relatively higher (~7.4), and benefits the adsorption of a neutral charged agent rather than a negatively charged agent.[29,30] According to this, the distinct accumulation of platinum in the spleen, kidney, heart and brain of the treated mice with the inherently neutrally charged oxaliplatin and the negatively charged PHDP is understandable.

3.3 Survival rate and heart/brain/blood platinum

The toxic reaction of the platinum agent in the organs and blood may ultimately affect the survival of the treated mice. After treating for ten consecutive days the survival rate of the 10 mice receiving both 16.7 and 167 μmol kg^{-1} of PHDP is 100%. This suggests that even at 167 μmol kg^{-1} dose, PHDP therapy still leads to a high survival rate. Besides, a similar survival rate (80%) was observed for the mice receiving

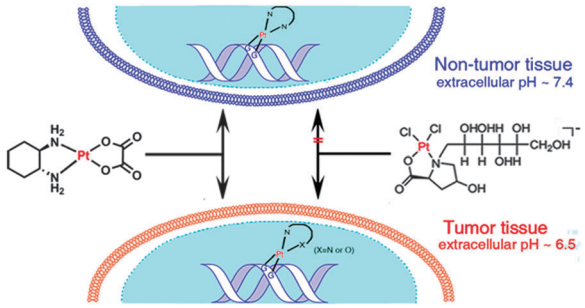

Fig. 11 A proposed scheme of mechanism of antitumor action of oxaliplatin and PHDP.

16.7 μmol kg^{-1} of oxaliplatin and 333 μmol kg^{-1} of PHDP. This means that the mouse mortality from 333 μmol kg^{-1} of PHDP is equal to that from 16.7 μmol kg^{-1} of oxaliplatin.

On the other hand, the accumulation of the platinum in the heart, brain and blood of the mice receiving 333 μmol kg^{-1} of PHDP is substantially equal to that of the mice receiving 16.7 μmol kg^{-1} of oxaliplatin. This means that the platinum toxicity of PHDP therapy to heart, brain and blood is 20 fold lower than that of oxaliplatin therapy. This could be attributed to the phenomena that the normal tissues are in favor of the distribution of neutrally charged oxaliplatin rather than negatively charged PHDP.[27–29]

Due to the therapies of 333 μmol kg^{-1} of PHDP and 16.7 μmol kg^{-1} of oxaliplatin having equal mortality and platinum accumulation in the heart, brain and blood, one could deduce that the platinum accumulation in the heart, brain and blood should be responsible for the death of platinum therapy.

3.4 Urine and fecal platinum

The platinum accumulated in the organs of the treated mice is believed to be eliminated *via* the urine and faeces. At a dose of 16.7 μmol kg^{-1}, the urine platinum of the mice receiving PHDP (152.36 μg of platinum per g of urine) is 1.2 folds higher than that of the mice receiving oxaliplatin (152.36 μg of platinum per g of urine) and 5.7 folds higher than that of the mice receiving cisplatin (26.78 μg of platinum per g of urine).[26] At this dose the urine quantity (2.56 mL per 24 h) of the mice receiving PHDP is 1.5 folds higher than that (1.66 mL per 24 h) of the mice receiving oxaliplatin. Therefore, within 24 h the platinum eliminated *via* the urine of PHDP treated mice is 1.8 folds higher than that of oxaliplatin treated mice. On the other hand, at this dose the faecal platinum (22.65 μg of platinum per g of feces) of PHDP treated mice is 1.5 fold higher than that (15.28 μg of platinum per g of feces) of oxaliplatin treated mice and 2.7 fold higher than that of the mice receiving cisplatin (8.36 μg of platinum per g of urine).[26] These differences between the elimination of PHDP and oxaliplatin *via* both urine and faeces are in agreement with the differences between the accumulation of PHDP and oxaliplatin in the organs and the blood of the treated mice.

4. Conclusion

The platinum accumulated in the spleen, kidney, heart, brain, blood, urine, faeces and tumor tissue of the mice receiving platinum agent is closely associated with its *in vitro* anti-proliferation activity, *in vivo* anti-tumor activity, *in vivo* toxic reaction, and the survival rate of the treated mice. The atomic absorption based determinations of the accumulation of the platinum in the spleen, kidney, heart, brain, blood, urine, faeces and tumor tissue of the treated mice constitute a model system that can be generally used in the investigation of the *in vitro* anti-proliferation activity, the *in vivo* anti-tumor activity and the toxic effects of the novel platinum agents. This model system proves that in respect to safety and efficacy, PHDP is better than oxaliplatin mainly due to its specificity to the tumor tissues and less specificity to non-tumor tissues, which result from its negative charge property (see Fig. 11).

5. Experimental

5.1 Reagents and instruments in chemical synthesis

All the reactions were carried out under nitrogen (1 bar). ^{1}H (300 and 500 M Hz) and ^{13}C (75 and 125 M Hz) NMR spectra were recorded on Bruker Avance II 300 MHz NMR and Avance II 500 MHz spectrometers for solution DMSO-d_6, or $CDCl_3$ with tetramethylsilane as the internal standard. IR spectra were recorded with a Perkin-Elmer 983 instrument. ESI/MS was determined on ZQ 2000 (Waters, US). Melting points were measured on a XT5 hot stage microscope (Beijing key electro-optic factory). Optical rotations were determined on a Schmidt and Haensch Polartronic D instrument at 20 °C. All L-amino acids and α-D-glucose were purchased from China Biochemical Corp. TLC was made with Qingdao silica gel GF_{254}. Chromatography was performed with Qingdao silica gel H_{60} or Sephadex-LH_{20}. All animals were purchased from the Experimental Animal Center of Capital Medical University. The study described herein was performed according to a protocol reviewed and approved by the ethics committee of Capital Medical University. The committee ensures that the welfare of the animals is maintained in accordance to the requirements of the Animal Welfare Act and according to the Guide for Care and Use of Laboratory Animals. All solvents were distilled and dried before use based on routine procedures.

5.2 Preparing PHDP

To the solution of 0.40 g (10 mmol) of NaOH in 3 mL of methanol/water (1 : 1), 1.31 g (10 mmol) of L-hydroxyproline was added. The mixture was stirred at room temperature for 20 min until it was completely dissolved. Then to this solution, 1.80 g (10 mmol) of D-glucose was added to the mixture and stirred at room temperature until D-glucose was completely dissolved. The solution was stirred at 50–60 °C for 5 h under an argon atmosphere at room temperature, treated with 1.62 g (30 mmol) of sodium borohydrade and stirred for another 96 h. The reaction mixture was cooled to 0 °C and adjusted to pH 2.5 by adding concentrated hydrochloric acid. The formed precipitates were removed by filtration and the filtrate was evaporated under vacuum. The residue was dilated with anhydrous ethanol and the precipitates that formed were removed by filtration. This procedure was repeated 5 times until the residue was dissolved in 10 mL of water. The solution was loaded on the column of acidic ion exchange resin and

eluted with 3% aqueous solution of *N*-methylmorpholine to give 652 mg (22%) of *N*-[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxylhex-1-yl]-L-hydroxyproline (PHDP) as a colorless powder. Mp 179–181 °C. IR (KBr): 3365, 2942, 2864, 1701, 1628, 1449, 1398, 1338, 1235, 1082, 925, 867. ESI (*m*/*z*): 296 $[M+H]^+$. ^{1}H NMR(300 MHz, D_2O) δ = 4.40(m, J = 4.7 Hz, 1H), 4.09 (m, J = 5.2 Hz, 1H), 3.85 (m, J = 5.0 Hz, 1H), 3.73 (m, J = 3.9 Hz, 1H), 3.63 (m, J = 5.2 Hz, 2H), 3.45 (m, J = 5.0 Hz, 1H), 3.36 (m, J = 4.9 Hz, 1H), 3.30 (m, J = 5.0 Hz, 1H), 3.22 (dd, J = 3.4 Hz, J = 12.3 Hz, 1H), 3.14 (t, J = 4.9 Hz, 1H), 3.10 (dd, J = 9.1 Hz, J = 12.4 Hz, 1H), 2.37 (m, J = 4.9 Hz, 1H), 2.10 (m, J = 4.9 Hz, 1H). Anal Calcd. for $C_{11}H_{21}NO_8$: C 44.74, H 7.17, N 4.74; Found: C 44.52, H 7.03, N 4.95.

The suspension of 295 mg (1.0 mmol) of PHDP and 415 mg (1.0 mmol) of K_2 [$PtCl_4$] in 10 mL of water was stirred at room temperature to form a clean solution. This solution was refluxed for 30 min, cooled to room temperature and filtered. The filtrate was evaporated under vacuum and the residue was crystallized in ethanol to give 480 mg (81%) of PHDP as a yellow powder. Mp 208–210 °C. IR (KBr): 3381, 2920, 2865, 1728, 1645, 1444, 1218, 1073, 879, 530. ESI-MS (*m*/*z*): 602 $[M+H]^+$. ^{1}HNMR (300 MHz, D_2O) δ = 4.37 (d, J = 4.6 Hz, 1H), 4.15 (m, J = 3.5 Hz, J = 7.0 Hz, 1H), 3.83 (m, J = 2.0 Hz, J = 5.5 Hz, 1H), 3.79 (m, J = 2.0 Hz, 1H), 3.48 (m, J = 5.0 Hz, 1H), 3.33 (m, J = 4.9 Hz, 1H), 3.30 (m, J = 5.0 Hz, 1H), 3.22 (dd, J = 3.4 Hz, J = 12.3 Hz, 1H), 3.11 (t, J = 4.9 Hz, 1H), 3.12 (dd, J = 9.1 Hz, J = 12.4 Hz, 1H), 2.32 (m, J = 4.9 Hz, 1H), 2.05 (m, J = 4.9 Hz, 1H). Anal Calcd. For $C_{11}H_{20}Cl_2KNO_8Pt$: C, 22.04; H, 3.36; N, 2.34; Found: C 22.22, H 3.49, N 2.46.

5.3 *In vitro* anti-proliferation assay

Different cancer cell lines (H1299, HL_{60}, HepG2, HeLa and MES-SA) were cultured at 37 °C in 75 cm^2 flask using RPMI1640 media (Gibco Laboratories, Santa Clara, CA, USA) containing 10% fetal calf serum (Gibco Laboratories), 100 U mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin. The cells were grown at a density of 1×10^5 cells mL^{-1} under humidity with 5% CO_2.

IC_{50} values of PHDP and oxaliplatin were determined in all cancer lines using using MTT assay as described previously (Mosmann, 1983). Briefly, cells were seeded at a density of 5×10^4 cells/well in a 100 μL volume of the medium in 96-well plates (Corning, NY, USA). Cells in 96-well plates were incubated for 48 h with serial dilution of each compound, then the plate was centrifuged at 1000 r/min for 10 min and the supernatant was aspirated. To each well containing residue 20 μL of MTT (5 g L^{-1}) was added. The 96-well plates were incubated at 37 °C for 4 h and the supernatant was aspirated. To each well containing the residue 100 μL of DMSO was added and then shaken for 8 min. The absorbance was measured at 570 nm (reference at 630 nm) on a 96-well microplate reader (Mode 680, Bio-Rad).

5.4 *In vivo* anti-tumor assay

The assessments described here were performed based on a protocol reviewed and approved by the ethics committee of Capital Medical University. The committee assures the welfare of the mice was maintained in accordance to the requirements of the animal welfare act and according to the guide for care and use of laboratory animals. Male ICR mice were maintained at 21 °C with a natural day/night cycle in a conventional animal colony. The mice were 10–12 weeks old at the beginning of the experiments. The tumor used is S180, which forms solid tumors, when injected subcutaneously. S180 cells for initiation of subcutaneous tumors were obtained from the ascitic form of the tumors in mice, which were serially transplanted once per week. Subcutaneous tumors were implanted by injecting 0.2 mL of 0.9% saline containing 2×10^6 viable tumor cells under the skin on the right oxter. Twenty-four hours after implantation, the mice were randomly divided into experimental groups. Thirteen mice of the positive control group were given a daily i.p. injection of 16.7 μmol kg^{-1} of oxaliplatin in 0.2 mL of 0.9% saline for ten consecutive days. Ten mice of the negative control group were given a daily i.p. injection of 0.2 mL of 0.9% saline for ten consecutive days. Ten mice of the treatment groups were given a daily i.p. injection of 16.7 μmol kg^{-1} or 166.7 μmol kg^{-1} of PHDP in 0.2 mL of 0.9% saline for ten consecutive days. Thirteen mice of the treatment group were given a daily i.p. injection of 333.0 μmol kg^{-1} of PHDP in 0.2 mL of 0.9% saline for ten consecutive days. After administration, the urine and fecal samples of each group were continually collected for 10 days. Twenty-four hours after the last administration, all mice were weighed and blood was drawn from the eye orbit. Then the mice were sacrificed by diethyl ether anaesthesia and dissected to immediately obtain and weigh the tumor, liver, kidney, brain, spleen, heart and left femur samples. All of the biosamples were digested in $HClO_4$ and HNO_3 (1 : 3) on a heating block, dried at 80 °C, redissolved in 1% nitric acid to determine the content of platinum using a Varian SpectrAA-110 atomic absorption spectrometer in the graphite furnace.

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