An integrin-targeted photoactivatable Pt(IV) complex as a selective anticancer pro-drug: synthesis and photoactivation studies†

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A new anticancer agent based on the conjugation of a photoactivatable Pt(IV) pro-drug to a cyclic RGD-containing peptide is described. Upon visible light irradiation, phototoxicity was induced preferentially in SK-MEL-28 melanoma cancer cells overexpressing αvβ3 integrin compared to control DU-145 human prostate carcinoma cells.

The use of visible light has enormous potential in chemotherapy for targeting, at a desired time, the place and dose, and release of cytotoxic species from inert anticancer pro-drugs. For this reason, much efforts has been dedicated to the development of photoactivatable metal-based anticancer complexes for improving drug efficacy and reducing toxic side-effects associated with platinum-based chemotherapeutic drugs currently used in the clinic.1 In addition, photoactivation offers the possibility for new mechanisms of action as well as the formation of novel adducts with the final biological target (not only DNA, but also RNA or proteins), which are important variables to overcome inherent or acquired resistance to cisplatin. Among photoactivatable metallo-drugs, Pt(IV) diazidodihydroxido complexes are particularly promising since they are inert and non-toxic in the dark, but become highly active against a range of cancer cell lines upon irradiation with visible light, including cisplatin-resistant cells (A2780cis).2 Such Pt(IV) pro-drugs accumulate in tumour cells and bind strongly to DNA by generating adducts distinct from those of cisplatin.3 Ru(II) arene complexes such as [η6-p-cym]Ru(bpm)(py)45 or its peptide derivatives can also be activated by visible light to induce the dissociation of the Ru-pyridine bond and the generation of an active species with capacity to react with DNA.4 A similar pro-drug approach has been described with some Ru polypyridyl complexes masked with thioether groups that can be removed selectively upon visible light irradiation.5 Very recently, caging groups have also been applied to control the activity of Ru(II) and Re(I) complexes.6

Despite these promising examples, it is desirable to improve some of the pharmacological properties of photoactivatable metallo-drugs, such as aqueous solubility and cell uptake, as well as higher selectivity against cancer cells. In this context, targeted approaches based on peptide vectors whose receptors are overexpressed on cancer cells in combination with light activation can be used to develop anticancer agents with a double mechanism of selectivity, such as the conjugates between a photoactivatable Ru(II) arene complex and receptor-binding peptides recently described by us51 or a conjugate between a caged Re(I) organometallic complex and bombesin.52

Herein we report the conjugation of a photoactivatable Pt(IV) pro-drug, trans,trans,trans-[Pt(N2)(OH)2(py)2] (1) (Fig. 1), to a cyclic peptide containing the RGD sequence (c-Arg-Gly-Asp), which is selectively recognized by αvβ3 and αvβ5 integrins. The overexpression of these transmembrane heterodimeric glycoproteins in different tumor cells together with their known relationship with tumor angiogenesis, which is an essential process for tumor growth and metastasis, make them relevant targets in medicinal chemistry.7 In fact, RGD-containing peptides have been exploited extensively for tumour imaging and for targeted drug delivery of cytotoxic compounds,8 including some metal-based anticancer drugs.9,10 As recently found by us for conjugates between a Pt(IV) derivative of picoplatin and RGD-containing peptides,11 we hypothesize that the peptide vector will confer complex 1 with selectivity for cancer cells overexpressing pro-angiogenic integrins such as αvβ3 and αvβ5. The novelty of this approach resides in the use of a photoactivatable Pt(IV) pro-drug since irradiation with visible light directly within the tumour will trigger the release of cytotoxic Pt(II) species from the internalized conjugate (3 in Fig. 1), thus providing a Pt(IV)-based anticancer agent with a dual control over selectivity.
integrins, and currently in clinical phase III trials for the treatment of patients with brain tumors. The incorporation of non-natural D-Phe and [N-Me]-Val in the cyclic structure is responsible for increasing both the stability in biological fluids and the higher selectivity for αvβ3 integrin over αvβ5 and αvβ1. In our case, replacement of N-methyl Val by Lys allowed further derivatization of the RGD-containing peptide with a polyethylene glycol spacer at the ε-NH2 function. Then, complex 2 was attached to peptide intermediate 4 by using HATU in the presence of DIPEA in anhydrous DMF for 2 h at RT in the dark. The expected Pt-c(RGDfK) conjugate (3) was obtained as a pale yellow solid (54% yield) after purification by reversed-phase HPLC and lyophilisation (Fig. S1, ESI†). Conjugate 3 was unambiguously characterized by high-resolution ESI mass spectrometry and 1H NMR spectroscopy. As shown in Fig. 3 and Fig. S2 (ESI†), a m/z value consistent with the calculated value of the charged species ([M+H]+) and with the expected isotopic mass distribution pattern of Pt was obtained. In addition, diagnostic signals from the platinum complex (pyridine ligands) and from the peptide moiety (amide NH protons and aromatic protons of D-Phe) in the aromatic region of the 1H NMR spectra confirmed the covalent attachment of the Pt complex to the peptide vector (Fig 3 and Fig S3, ESI†).

Next, the efficiency of the photoactivation of Pt-c(RGDfK) conjugate (3) in the presence of 5’-GMP (2 mol equiv.) was investigated by HPLC-MS. As shown in Fig. S4 (ESI†), irradiation (λex= 420 nm, 11 mW cm⁻², 45 min, 37°C) led to the complete disappearance of 3 and to the formation of a major species that was characterized by HR-ESI-MS as the Pt(II)-GMP adduct, trans-[Pt(N3)(5’-GMP)(py)2]⁺ (6 in Fig. 2) (GMP is considered neutral in all the formulae). In addition, two minor GMP adducts were identified, trans-[Pt(py)2(5’-GMP)]⁺ and [Pt(N3)(py)(5’-GMP)]⁺. The photodissociation of conjugate 3 to form the Pt(II)-GMP adduct as a major product, parallels the behaviour observed for the parent complexes 1 and 2, indicating that the attached peptide does not alter the photochemical properties or the type of photoadducts with a model nucleobase. Furthermore, the release of the intact succinate-c(RGDfK) moiety (5 in Fig. 2), implies that the carrier ligand neither competes with 5’-GMP for binding to the photorelease Pt(II) species, nor does it form any secondary reactions, being a simple targeting vector of the Pt(IV) pro-drug.

Having established the photoactivation properties of the Pt-c(RGDfK) conjugate, our next objective was to investigate its toxicity towards different cancer cell lines in the presence of visible light to assess the capacity of the peptide vector to deliver the photoactivatable Pt(IV) pro-drug into cancer cells overexpressing integrin receptors. On the basis of flow cytometry studies (Fig. 4), we selected SK-MEL-28 human malignant melanoma cell line as a model to evaluate the phototoxicity of 3 since it expresses high levels of αvβ3 integrin compared with αvβ5 integrin (mean cell fluorescence intensity of 21.7 and 23.3 for αvβ3 and αvβ5 integrins, respectively). As negative control, the DU-145 human prostate carcinoma cell line was selected since the expression of αvβ3 integrin was considerably lower, whereas that of αvβ5 integrin was similar (mean cell fluorescence intensity of 16.6 and 31.8 for αvβ3 and αvβ5 integrins, respectively). As expected, the internalization of the fluorescein-labeled RGD-containing peptide, Fluo-c(RGDfK) (7), was slightly higher in the αvβ3 integrin overexpressing SK-MEL-28 cells than in DU-145 (by 1.6-fold when incubated at 10 μM; see Fig. S5, ESI†), which points to the active participation of this integrin receptor in the uptake of the peptide.

The photocytotoxicity of the Pt-c(RGDfK) conjugate (3) and of the parent Pt complexes (1 and 2) was determined upon irradiation with visible light (λex= 420 nm, 5 J/cm²) in both cell lines. The photoactivated dose-dependent inhibition of cell viability for compounds 1-3 towards SK-MEL-28 and DU-145 cells and their photoxic indices are summarised in Table I and the cytotoxicity plots are shown in Fig. S6 (ESI†). First, it is worth noting that the IC50 value for complex 1 in SK-MEL-28 was similar to those previously found in other cancer cell lines (IC50 = 6.8 μM in HaCaT, 8.3 μM in A2780 and 8.4 μM in OE19, under blue light irradiation), although the cytotoxicity in DU-145 cells was about 4-fold lower than in the melanoma cancer cell line. Hence, these results confirm the high antitumour efficiency of this Pt(IV) pro-drug against cancer cells of different origin when photovacatived with visible light. Second, the
cytotoxic effect of 1 was slightly reduced in SK-MEL-28 (about 1.5-fold) upon derivatization with a succinate group (2). This tendency was not reproduced in DU-145, since the cytotoxic effect was increased upon succinylation (IC\(_{50}\) =20 µM for 2 vs IC\(_{50}\)=43 µM for 1).

Since the cellular uptake of the Pt-peptide conjugate depends both on the level of expression of the pro-angiogenic integrins and on the binding affinity of the RGD-containing peptides towards these receptors, the determination of the intracellular accumulation is of high importance to assess the effect of the peptide conjugation on the biological activity of the Pt(IV) pro-drug as well as to investigate the contribution of each integrin subtype. For this purpose, in addition to SK-MEL-28 and DU-145 cells, we selected the MBA-MD-468 breast adenocarcinoma cell line as positive control for α\(_{v}\)β\(_{3}\) integrin. As shown in Fig. 4, the expression of α\(_{v}\)β\(_{3}\) integrin was considerably higher than that of α\(_{v}\)β\(_{5}\) integrin (mean cell fluorescence intensity of 3.8 and 42.4 for α\(_{v}\)β\(_{3}\) and α\(_{v}\)β\(_{5}\) integrins, respectively). Then, the three cancer cell lines were exposed to 10 µM solutions of compounds 1-3 in the dark for 1 h, and the intracellular level of platinum was quantified by inductively-coupled plasma mass spectrometry (ICP-MS).

As shown in Fig. 5, the accumulation of platinum after exposure of the three cell lines to Pt-c(RGDfK) conjugate (3) (46.6 ± 2 pmol Pt/10\(^6\) cells in DU-145, 130.7 ± 9 pmol Pt/10\(^6\) cells in SK-MEL-28 and 166.5 ± 6 pmol Pt/10\(^6\) cells in MBA-MD-468) was higher than that of complex 1 (26.2 ± 2.4 pmol Pt/10\(^6\) cells in DU-145, 72.6 ± 1.9 pmol Pt/10\(^6\) cells in SK-MEL-28 and 73.6 ± 2 pmol Pt/10\(^6\) cells in MBA-MD-468) or 2 (15.9 ± 2.7 pmol Pt/10\(^6\) cells in DU-145, 75.4 ± 9.6 pmol Pt/10\(^6\) cells in SK-MEL-28 and 86.8 ± 7 pmol Pt/10\(^6\) cells in MBA-MD-468). This clearly indicates that peptide conjugation has a positive effect on the intracellular accumulation of the photoactivatable Pt(IV) pro-drug. Notably, platinum accumulation in SK-MEL-28 cells after exposure to conjugate 3 was higher (about 2.5-fold) than in DU-145 cells, which agrees with the higher expression of α\(_{v}\)β\(_{3}\) integrin in the human malignant melanoma cell line compared with the prostate carcinoma cell line, as well as with the internalization studies with the fluorescein-labelled peptide.
and αβ3 integrins and suggest in all cases the participation of the peptide in the internalization of the conjugate. The reduced selectivity of conjugate 3 for αβ3 integrin compared with Cilengitide can be attributed to the replacement of [N-Me]-Val by the Lys residue where the photactivatable Pt(IV) complex is attached. Hence, on the basis of the overall results, we can envisage the integrin-mediated internalization and accumulation of the intact Pt-peptide conjugate in cancer cells overexpressing αβ3 and/or αβ3 integrins, where it will be photovativated to generate cytotoxic Pt(II) species with capacity to react with nucleic acids, as inferred by the adduct generated with 5'-GMP. Otherwise, a premature activation of the Pt(IV) pro-drug or hydrolysis of the conjugate would lead to similar or even lower Pt accumulation ratios than those obtained with control complexes.

Interestingly, a correlation was found between intracellular accumulation of conjugate 3 and phototoxicity (see Table 1): a lower IC50 value upon visible light irradiation and a higher phototoxic index was found in the melanoma cancer cells that accumulated a higher amount of the compound compared with prostate carcinoma cells. Notably, the accumulation of 1 and 2 in SK-MEL-28 and MBA-MD-468 cells was also higher than in DU-145, thereby revealing a preference for the melanoma and breast cancer cells. It is also interesting that despite the higher accumulation of conjugate 3 compared with the parent complexes, the phototoxicity was slightly reduced, particularly when comparing with 1 in SK-MEL-28. This might be attributable to differences in the quantum yield of the compounds and to the accumulation of the conjugate in intracellular vesicles that might interfere with the interaction of the released Pt(II) species with the target.

In conclusion, our results demonstrate the potential of conjugating photactivatable metal complexes, such as Pt(IV) pro-drugs, to peptides with the aim of generating receptor-targeted metal-based anticancer drugs with reduced toxic side effects based on dual control over selectivity. The fact that the Pt-c(RGDfK) conjugate can also be internalized by αβ3 integrin opens the door to delivering such promising anticancer metallodrugs to tumours overexpressing αβ3 integrin13 or to tumours coexpressing both αβ3 and αβintegrins.76,14 Such a multi-integrin targeting approach would provide new metal-based anticancer strategies and so benefit a wider range of patients by increasing the number of tumours which can be targeted.15

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Notes and references

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