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CISPLATIN EFFECT ON HEMOGLOBIN AND MYOGLOBIN AUTOOXIDATION

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ABSTRACT. It has previously been shown using mass-spectrometry that therapeutically-useful platinum-based compounds of the cisplatin family bind to a range of proteins, including hemoglobin and cytochrome *c*. On the other hand, currently available data suggests that the medically-relevant chemical properties of hemoglobin are in no significant way affected by Pt-based drugs, within the concentration ranges attainable during treatment of a patient. Here, cisplatin is shown to modulate two physiological parameters of hemoglobin and myoglobin - autooxidation and oxygen dissociation rate, as measured by UV-vis aspectra. The extent, to which these changes in reactivity impact the side-effects and even the therapeutic mechanisms of these drugs, appears to deserve further attention.

Keywords: *hemoglobin, cytochrome c, cisplatin, autooxidation*

INTRODUCTION

Cisplatin and related compounds are known to exert much of their useful therapeutic effects via binding to DNA.[1] The need for more effective drugs as well as the wide range of side-effects (nausea, progressive peripheral sensory neuropathy, fatigue, vomiting, alopecia, hematological suppression, renal damage) have, for several decades now, fuelled interest into understanding the complex mechanisms of interaction of cisplatin and related compounds with various biomolecules.[2,3] One notable observation in this respect has been that platinum can bind to a range of proteins, as demonstrated by elemental analyses, chromatography and mass spectrometry.[3,4] With such methods, cisplatin–Hb complexes were shown to be formed using clinically relevant concentrations of cisplatin and Hb. The interaction of oxaliplatin and carboplatin with hemoglobin was also studied with nanoelectrospray ionization quadrupole time-of-flight mass spectrometry (nanoESI-QTOF-MS) and size-exclusion high performance liquid chromatography/inductively coupled plasma mass spectrometry (HPLC/ICPMS) [5], showing similar results to those obtained with cisplatin; heme release was a noted side effect of platinum binding. Cisplatin-derived platinum was also found bound to cytochrome, with a preference for the iron ligand Met 65.[6]

Hb is present in high concentrations in blood and is particularly sensitive to changes in redox status, to the extent that under stress conditions such as physical effort or certain pathological conditions it engages in toxic reactions

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with oxidative stress agents- primarily peroxide – yielding free radicals and highly-oxidizing states at the iron (ferryl, Compound II).[7-10] As such, it may be expected that Hb might be sensitive to the stress imposed by cisplatin in patients; indeed, in a preliminary report we have shown that the autooxidation rate of hemoglobin is affected by cisplatin and related compounds.[11] Cytochrome *c* not only serves as key component of the electron transport chain, but is also involved, most likely via redox reactions once again linked to peroxides, in the apoptosis process and as such would appear as a sensitive target for exogenous compounds such as cisplatin.[12-14] Here, data is shown establishing a link between previously-demonstrated binding of platinum to hemoglobin and myoglobin, and their reactivity towards dioxygen with possible relevance to the *in vivo* reactions of platinum-based drugs

RESULTS AND DISCUSSION

Table 1 illustrates that, in agreement with a previous preliminary report,[11] cisplatin enhances the rate of autooxidation of hemoglobin, as measured under two different conditions – at 37ºC in pH 7.4 buffer or at room temperature under acceleration by guanidinium hydrochloride. The latter procedure is interpreted to effectively probe the stability of the protein: binding of platinum to the protein surface (previously proven by others using mass spectrometry [5]) is expected to affect the local mobility of the protein side-chains and hence the stability of the tertiary structure. As seen in Table 1, guanidine accelerates autooxidation; this is proposed to be due to partial denaturation of the protein induced by guanidine, which would result in opening the heme site towards solvent. Indeed, solvent accessibility and polarity at the metal site is a key parameter dictating autooxidation parameters in dioxygen carriers.[15] Under these conditions, it was expected that in the presence of guanidine the Pt-treated Hb would show slightly increased autooxidation – as confirmed by the data in Table 1. One may note, from a methodological point of view, that our approach of measuring globin autooxidation rate in the presence of a controlled amount of guanidine offers the advantage of measuring this rate somewhat faster than traditionally done at 37ºC in phosphate buffer saline. While the absolute values obtained for autooxidation in the presence of guanidine at room temperature (shown in Table 1) lack direct physiological relevance, they do allow for a faster way of assessing the effect of exogenous factors on the autooxidation rate and on stability in general - such as exemplified here by the cisplatin case. Indeed, while traditional measurements of autooxidation rates in dioxygen-carrying proteins, done at 37ºC and in PBS, may take several hours, the guanidine procedure requires no incubation and can be controlled, by choosing the appropriate guanidine concentrations, to proceed in only a few minutes.

Table 2 illustrates that cisplatin affects the rate of dioxygen dissociation from the hemoglobin ferrous heme (k_{off}). The 14-26% decreases brought by cisplatin compared to native Hb may be interpreted to suggest slightly reduced mobility of protein side-chains controlling dioxygen liberation. With myoglobin

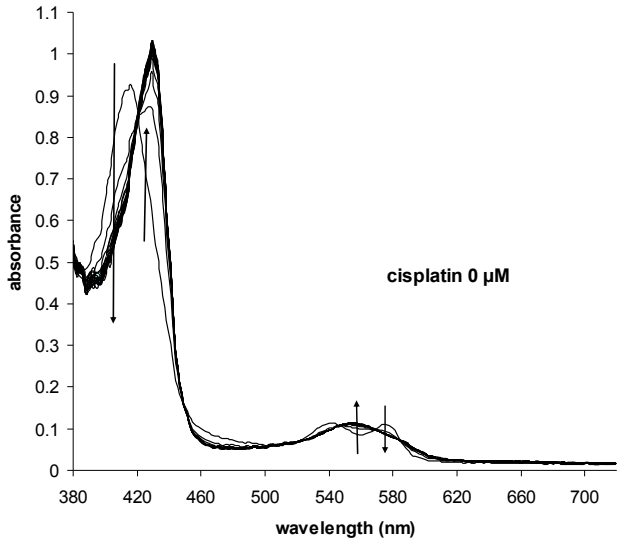
the k_{off} rate was affected somewhat similar to hemoglobin (data not shown). Figure 1 illustrates UV-vis spectra collected during data collection for k_{off} measurements.

Table 1. The effect of cisplatin on Hb and Mb autooxidation rate as induced by temperature or by 2.5 M guanidine. Percentage increases or decreases relative to values seen for the respective proteins in the absence of cisplatin are shown.

	Hb (%)	Mb (%)
100 μM Pt	+ 2 (±1)	+6 (±1)
300 μM Pt	+ 34 (± 1)	+11 (±6)
30 μM Pt, guanidine	+48 (± 13)	
100 μM Pt, guanidine	+13 (± 5)	
300 μM Pt, guanidine	+33 (± 1)	
500 μM Pt, guanidine	+36 (± 1)	

Table 2. Effect of cisplatin on the dissociation rate of dioxygen from oxy-Hb. Percentage increases or decreases relative to values seen for Hb in the absence of cisplatin are shown.

	k_{off}
Hb, 200 μM Pt, inc	-23%±0.1
Hb, 200 μM Pt, non-inc	-26%±0.1
Hb, 400 μM Pt, inc 24 h (mM)	-14%+0.1



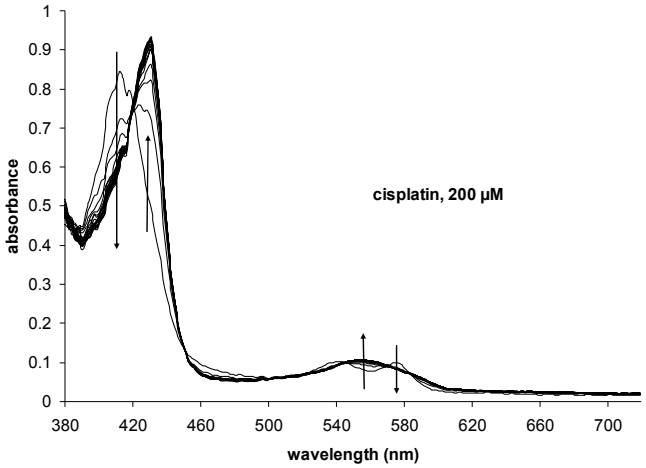


Figure 1. UV-vis spectra collected during a stopped-flow experiment where oxy-hemoglobin (14 µM) was mixed with dithionite (20 mM) in order to monitor the transition from the oxy to the deoxy state, in the presence or absence of 200 µM cisplatin. Spectra were collected every 13 ms for the first 2 seconds after mixing; arrows indicate the directions in which absorbance changes at various wavelengths.

CONCLUSIONS

Cisplatin affects the autooxidation rate and the oxygen dissociation rate in hemoglobin, more so than in myoglobin. The rate of dissociation of molecular oxygen from ferrous hemoglobin is, by contrast, slightly lowered by cisplatin. Together, these findings suggest that cisplatin is likely to limit the dioxygen-delivering abilities of hemoglobin. This is proposed to originate in a decrease in flexibility of the protein, as a result of platinum binding to the surface aminoacids. It remains to be established to what extent these findings have a clinically-relevant outcome.

METHODS

Bovine hemoglobin was purified from bovine blood as previously described.[16] Thus, the blood, freshly drawn on citrate, was centrifuged 15 minutes at 5000 rpm to separate the red blood cells, which were then washed three times with 5 mM phosphate pH 7.4 + 150 mM NaCl. Hemoglobin concentrations in text are given per heme rather than per tetramer. The met forms of the hemoglobins were prepared by ferricyanide treatment as previously described.[17-19] Where needed, guanidinium hydrochloride was added to reaction mixtures from 6 M stock solutions.

UV-vis spectra were recorded on Agilent 8453 (Agilent, Inc.) and Cary 50 (Varian, Inc) instruments. Stopped-flow spectra were collected on a Biologic SFM-300 system equipped with 3 syringes and capable of sequential mixing,

with a high-speed diode array detector. Stopped-flow data were analyzed within the SPECFIT32 software package (BioLogic Science Instruments, France) using Singular Value Decomposition (SVD) and global multiexponential fitting of the SVD treated data, with the spectra fitted to simple kinetic models using Levenberg-Marquardt or Simplex algorithms.

Autooxidation rates were measured at 37° C in PBS. Optionally, prior to measurements the samples were incubated at 4ºC overnight, with physiological serum (control sample) or with cisplatin dissolved in physiological serum at concentrations indicated in the Table 1. Alternatively, autooxidation measurements were measured at room temperature in the presence of 2.5 M guanidine, in which case pre-incubation with cisplatin did not affect the result.

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REFERENCES

1. S.E. Sherman,S.J. Lippard, *Chem. Rev.*, **1987**, *87*, 1153
2. J. Peng, R. Mandal,M. Sawyer, X. F. Li, *Clin. Chem.*, **2005**, *51*, 2274
3. R. Mandal, C. Teixeira, X. F. Li, *The Analyst*, **2003**, 629
4. A. Casini, A. Guerri,C. Gabbiani, L. Messori, *J. Inorg. Biochem.*, **2008**, *102*, 995
5. R. Mandal, M.B. Sawyer, X.F. Li, *Rapid Commun. Mass Spectrom.*, **2006**, *20*, 2533
6. T. Zhao, F.L. King, *J. Am. Soc. Mass. Spectrom.*, **2009**, *20*, 1141
7. R. Silaghi-Dumitrescu, B.J. Reeder, P. Nicholls, C.E. Cooper, M.T. Wilson, *Biochem. J.*, **2007**, *403*, 391
8. N.B. Vollaard, J.P. Shearman, C.E. Cooper, *Sports Med.*, **2005**, *35*, 1045
9. N.B. Vollaard, B.J. Reeder, J.P. Shearman, P. Menu, M.T. Wilson, C.E. Cooper, *Free Radic. Biol. Med.*, **2005**, *39*, 1216
10. B.J. Reeder, D.A. Svistunenko, C.E. Cooper, M.T. Wilson, *Antioxidant & Redox Signaling*, **2004**, *6*, 954
11. V. Taciuc, C. Bischin, R. Silaghi-Dumitrescu "A novel mechanism for platinum-based drugs: cisplatin and related compounds as pro-oxidants in blood", Silaghi-Dumitrescu, R. and Garban, G., Ed.; Cluj University Press: Cluj-Napoca, Romania, **2009**, pp 130
12. V.E. Kagan, G.G. Borisenko, Y.Y. Tyurina, V.A. Tyurin, J. Jiang, A.I. Potapovich, V. Kini, A.A. Amoscato, Y. Fujii, *Free Radic. Biol. Med.*, **2004**, *37*, 1963

13. V.E. Kagan, V.A. Tyurin, J. Jiang, Y.Y. Tyurina, V. B. Ritov, A.A. Amoscato, A.N. Osipov, N.A. Belikova, A.A. Kapralov, V. Kini, Vlasova, II, Q. Zhao, M. Zou, P. Di, D.A. Svistunenko, I.V. Kurnikov, G.G. Borisenko, *Nat. Chem. Biol.*, **2005**, *1*, 223
14. V.E. Kagan, H.A. Bayir, N.A. Belikova, O. Kapralov, Y.Y. Tyurina, V.A. Tyurin, J. Jiang, D.A. Stoyanovsky, P. Wipf, P.M. Kochanek, J.S. Greenberger, B. Pitt, A.A. Shvedova, G. Borisenko, *Free Radic. Biol. Med.*, **2009**, *46*, 1439
15. D.M. Kurtz, Jr., *Ess. Biochem.*, **1999**, 55
16. E. Antonini, M. Brunori, "Hemoglobin and Myoglobin in their Reaction with Ligands", North-Holland, Amsterdam, 1971.
17. B.J. Reeder, D.A. Svistunenko, M.A. Sharpe, M.T. Wilson, *Biochem.*, **2002**, *41*, 367
18. J. Dunne, D.A. Svistunenko, A.I. Alayash, M.T. Wilson, C.E. Cooper, *Adv. Exp. Med. Biol.*, **1999**, *471*, 9
19. J. Dunne, A. Caron, P. Menu, A.I. Alayash, P.W. Buehler, M.T. Wilson, R. Silaghi-Dumitrescu, B. Faivre, C.E. Cooper, *Biochem. J.*, **2006**, *399*, 513