Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 363 (2007) 235–240
BBRC
www.elsevier.com/locate/ybbrc

DNA damage induced by novel demethylcantharidin-integrated platinum anticancer complexes

Siu-Kwong Pang [a,b], Chun-Wing Yu [a], Steve C.F. Au-Yeung [b], Yee-Ping Ho [a,*]

[a] School of Pharmacy, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China
[b] Department of Chemistry, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China

Received 28 August 2007
Available online 7 September 2007

Abstract

Oxaliplatin is a third generation platinum (Pt) drug with a diaminocyclohexane (DACH) entity, which has recently obtained worldwide approval for the clinical treatment of colon cancer, and apparently operates by a different mechanism of action to the classical cisplatin or carboplatin. Introducing a novel dual mechanism of action is one approach in designing a new platinum-based anticancer agent, whereby an appropriate ligand, such as demethylcantharidin (DMC), is released from the parent compound to exert a cytotoxic effect, in addition to that of the DNA-alkylating function of the platinum moiety. To investigate the likelihood of a novel dual mechanism of anticancer action, demethylcantharidin-integrated Pt complexes: Pt(*R,R*-DACH)(DMC) with the same Pt–DACH moiety as oxaliplatin, and $Pt(NH_3)_2$(DMC) akin to carboplatin; were studied for their ability to induce DNA damage in HCT116 colorectal cancer cells by an alkaline comet assay. The results showed that the DMC ligand released from the novel complexes caused additional DNA lesions when compared with oxaliplatin and carboplatin. The comet assay also revealed that the DNA-damaging behavior of cisplatin is characteristically different; and this study is the first to demonstrate the ability of DMC to induce DNA lesions, thus providing sufficient evidence to explain the superior antiproliferative effect of the novel DMC-integrated complexes.

Keywords: Demethylcantharidin–platinum complexes; DNA damage; Alkaline comet assay

Cisplatin is a classical Pt-based anticancer drug that is widely used for the treatment of a broad spectrum of tumors [1]. However, its use has been limited by side effects such as nephrotoxicity and neurotoxicity, and acquired and intrinsic resistance. Carboplatin is a second generation platinum anticancer drug that exhibits a similar biological profile to cisplatin but of lower potency and reduced toxicity [1]. More recently, oxaliplatin has been found to be active against colorectal cancer, which is intrinsically resistant to cisplatin (Fig. 1) [2]. The cytotoxicity of oxaliplatin is comparable to and occasionally greater than that of cisplatin [2], but neurotoxicity is significant [1].

The cytotoxicity of classical platinum compounds is attributed to the formation of Pt–DNA adducts through the platination at N7-sites of purine bases in DNA, giving rise to 1,2-d(GpG) intrastrand cross-links [3]. In addition, 1,3-d(GpXpG) intrastrand cross-links are also a major product of carboplatin-induced DNA lesions [4]. The futile-cycling model involving the mismatch repair pathway (MMR) is generally accepted as an explanation for the cytotoxicity induced by cisplatin–DNA adducts [2]. This concept has been reinforced by the recent discovery of an MMR-dependent apoptotic activation mechanism [5].

Oxaliplatin forms *R,R*-DACH–Pt–DNA adducts (*R,R*-DACH: *trans*-*R,R*-diaminocyclohexane) via platination at DNA resulting in 1,2-d(GpG) intrastrand cross-links [6]. In contrast to cisplatin–DNA adducts, the MMR protein hMutSα has a low affinity for oxaliplatin–DNA adducts [2]. The hydrolysis of oxaliplatin is also slower [7], and as a consequence, forms fewer Pt–DNA adducts than cisplatin. Both cisplatin– and oxaliplatin–DNA adducts are removed to a similar extent by the NER mechanism [2], but the latter show a lower affinity for HMGB1, which

* Corresponding author. Fax: +852 2603 5295.
E-mail address: yeepingho@cuhk.edu.hk (Y.-P. Ho).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.08.172

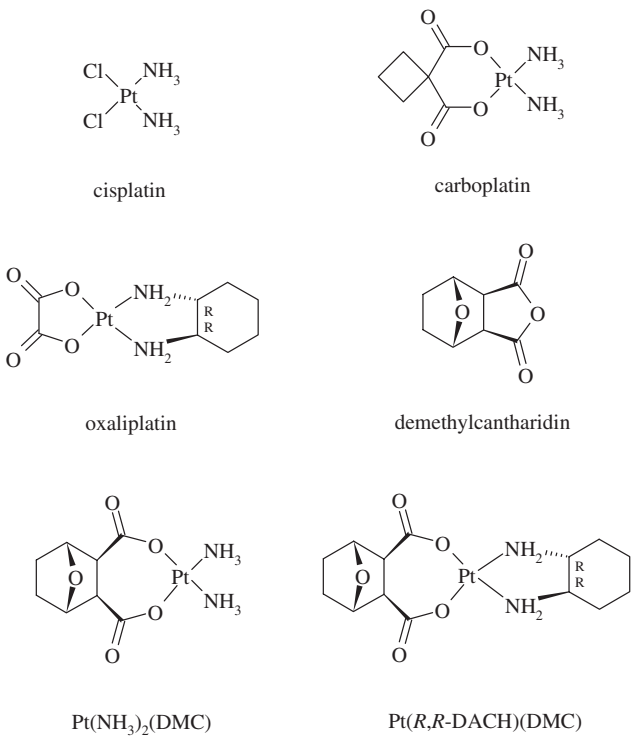

Fig. 1. Chemical structures of compounds referred to in this study.

shields the NER proteins in repairing DNA lesions caused by platinum compounds [8]. Therefore, an unique mechanism of drug action originating from the Pt(*R,R*-DACH) moiety has been postulated that is different from cisplatin [2].

Demethylcantharidin (norcantharidin) is a demethylated form of cantharidin, which is an active principle of *Mylabris* (dried body of the blister beetle) that has been used as a traditional Chinese medicine (TCM) [9]. Both cantharidin and demethylcantharidin (DMC) possess anticancer activity [10], and the latter, being less toxic, has been used to treat hepatoma, oesophagus carcinoma, and gastric cancer [9]. Previous studies showed that cantharidin and DMC inhibited the activity of protein phosphatases, particularly PP2A, and were able to promote the cell cycle from G_1 to S phase with subsequent G_2/M arrest [11]; and it has been suggested that as a result, cells died from immature mitosis [10]. A recent study has demonstrated that cantharidin can cause DNA damage, which may be the main contributory factor for the cytotoxicity of DMC and cantharidin [12]; but the actual mechanism of action remains unclear.

We have reported the synthetic integration of DMC with a *trans-R,R*-diaminocyclohexane platinum moiety (*R,R*-DACH–Pt), that is akin to oxaliplatin; and a diamine platinum moiety ($(NH_3)_2$Pt), akin to carboplatin [11] (Fig. 1). Our studies have shown that Pt(*R,R*-DACH)(DMC) exhibited greater cytotoxicity than cisplatin in colon cancer cell lines, and flow cytometric analysis indicated that this compound behaved similarly to oxaliplatin

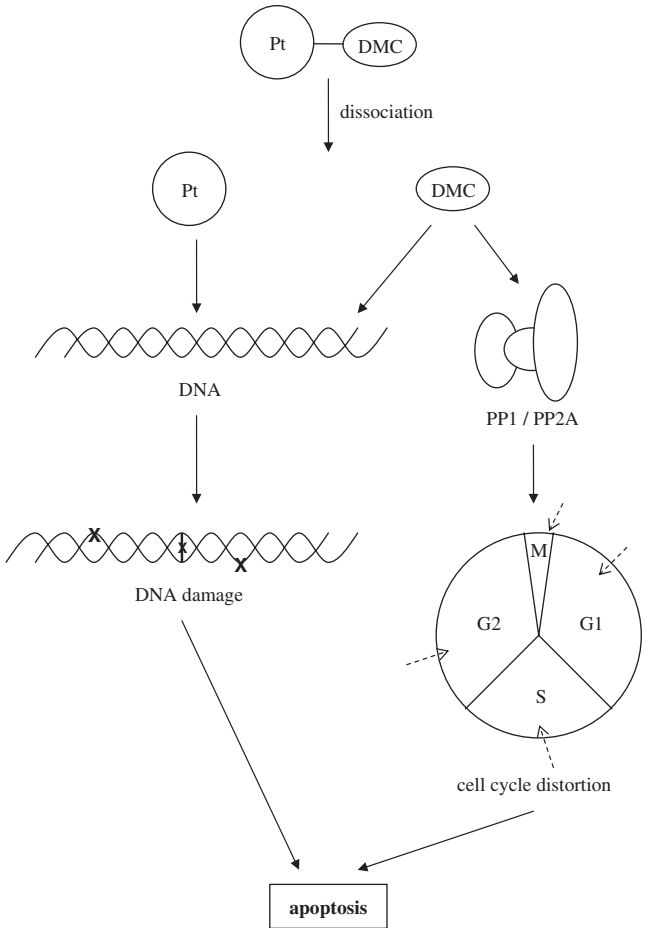

Fig. 2. Schematic presentation of a dual mechanism of anticancer action exerted by DMC–platinum integrated complexes.

in influencing the cell cycle of HCT116 (human colon cancer) cells [11]. Correspondingly, $Pt(NH_3)_2$(DMC) was more potent than carboplatin, and an early induction of apoptosis was observed despite the compounds producing similar cell cycle distributions [11].

Therefore, based on our previous findings, it is possible that a novel dual mechanism of drug action for the DMC–Pt complexes exist, whereby: (i) the platinum moiety alkylates DNA (as do classical Pt drugs); and (ii) the leaving ligand (DMC) induces an extra cytotoxic effect (by additional DNA damage and/or cell cycle distortion) in cancer cells (Fig. 2) [13].

The purpose of this study was to verify the DNA damage caused by the released DMC ligand from the novel compounds, by utilizing an alkaline comet assay and determining the Olive tail moments (OTM); and graphite furnace atomic absorption (GFAA) spectroscopy, to determine the level of Pt–DNA adducts formed. Flow cytometry was used to ensure that the DNA strand breaks detected by the alkaline comet assay were not due to apoptosis and/or necrosis. The HCT116 colorectal cancer cell line was selected because of its MMR-deficiency contributing to intrinsic cisplatin-resistance.

Materials and methods

Chemicals and reagents. Cisplatin and carboplatin were purchased from Strem Chemicals, Inc. (Newburyport, USA), and oxaliplatin was supplied by W.C. Heraeus GmbH & Co. KG (Hanau, Germany). DMC was synthesized from a Diel–Alder's reaction between furan and maleic anhydride, and $Pt(NH_3)_2$(DMC) and Pt(*R,R*-DACH)(DMC) were synthesized according to procedures described previously [11].

Cell culture and drug treatment. HCT116 cells from the American Type Culture Collection (ATCC, Rockville, MD, USA) were grown in RPMI 1640 medium, according to standard protocols. Cells were plated in tissue culture dishes and incubated (37 °C, 5% CO_2) for 18–24 h before drug treatment. Cisplatin, carboplatin, oxaliplatin, DMC, $Pt(NH_3)_2$(DMC), and Pt(*R,R*-DACH)(DMC) (50 μM) were exposed to cells for 3 h. Untreated cells were used as the control.

Alkaline comet assay. The alkaline comet assay was performed according to published procedures with some modifications [14]. Cells (approximately 250,000) treated with drug were trypsinized and suspended in 1 mL (0.5%) low melting point agarose (LMPA). A 75 μL cells–LMPA mixture was layered onto frosted microscope slides, which were pre-coated with 1% normal agarose. After solidification at 4 °C for 30 min, the embedded cells were lysed at 4 °C overnight (2.5 M NaCl, 10 mM Tris, 100 mM EDTA, and 1% Triton X-100, pH 10). The slides were incubated in an alkaline electrophoresis solution (1 mM EDTA, 300 mM NaOH, pH > 13) at 4 °C for 40 min; followed by electrophoresis (2 V/cm) at 4 °C for 30 min and washed with neutralization buffer (0.4 M Tris, pH 8) three times before immersion in absolute ethanol for 20 min, and air dried at room temperature. The slides were then stained with ethidium bromide (20 μg/mL) for 10 min and observed with a fluorescence microscope (Nikon ECLIPSE E600). Fifty cells were scored onto each slide and their Olive tail moments were measured using Komet 3.1 Europe software. The analysis was repeated in triplicate.

Determination of sub-G_1 population. Flow cytometry was employed for the sub-G_1 population measurements [15]. Approximately 1×10^6 cells were seeded. After drug treatment, cells were trypsinized and fixed in 70% ethanol until analysis. Ethanol was removed before cells were suspended in 1 mL staining solution (10 mL PBS containing 10 μL Triton X-100, 2 mg DNase-free RNase A and 200 μL of 1 mg/mL propidium iodide) for 1 h in the dark at room temperature. Each experiment was performed with 20,000 events with three replicates on a FACScan EPICS® ALTRA™ flow cytometer (Miami, USA). The sub-G_1 population was quantitated using WinMDI 2.8 software.

Quantitation of Pt–DNA adducts. $1–20\times10^7$ cells were seeded, depending on the level of DNA platination. A modified phenol–chloroform method was used for the extraction of DNA [16]. Briefly, cells were trypsinized and digested (100 mM NaCl, 10 mM Tris, 25 mM EDTA, 0.5% SDS, 0.1–1 mg/ml proteinase K (cell number dependent), pH 8, 50 °C, 48 h). The phenol/chloroform/isoamyl alcohol mixture (25:24:1 by volume) was added, and the aqueous layer was collected. 0.1% SDS and 1 μg/mL DNase-free RNase were used for removing RNA at 37 °C for 1 h. DNA was precipitated with 7.5 M ammonium acetate and absolute ethanol, rinsed with 70% ethanol, and air dried at room temperature. The DNA pellet was dissolved in distilled water at 37 °C. A portion was mixed with TE buffer (10 mM Tris, 1 mM EDTA, pH 8) for DNA quantitation. The remaining portion was mixed with a 7% w/v nitric acid (1:1 by volume), and incubated at 70 °C for 18 h for analysis of platinum content [17]. DNA concentration was measured as absorbance at 260 nm on a Perkin-Elmer UV/Vis Spectrometer Lambda Bio 40, after diluting the DNA sample with TNE buffer (100 mM Tris, 10 mM EDTA, 2.0 M NaCl, pH 8). The platinum content was determined by GFAA on an Hitachi Z-8200 atomic absorption spectrometer. At least three independent experiments were performed.

Statistical analysis. For the alkaline comet assay; the determination of Pt–DNA adduct levels; and the measurement of sub-G_1 populations; results were expressed as means ± standard deviation for replicate experiments. Data for the alkaline comet assay were analyzed using one-way ANOVA using SPSS 14.0 followed by the post-hoc LSD (least square difference) test. The significance differences of Pt–DNA adduct levels between two kinds of treatment were determined by a *t*-test.

Results

DNA damage induced by various drug treatments

DNA lesions induced by the test compounds on HCT116 cells were detected by an alkaline comet assay and the OTMs measured (Fig. 3). When the three platinum drugs were compared, the tail moment for cisplatin treatment was reduced when compared with the control (3.7 *vs.* 8.4; $P < 0.05$), and it was the shortest among the compounds tested. In contrast, there were increased tail moments after carboplatin and oxaliplatin treatments (oxaliplatin: 23.7; carboplatin: 17.4; control: 8.4; $P < 0.05$); and that for DMC was intermediate (18.5). The tail moments for $Pt(NH_3)_2$(DMC) and Pt(*R,R*-DACH)(DMC) were increased relative to the control (29.1; 39.1; 8.4, respectively; $P < 0.05$). The order of OTM was as follows:

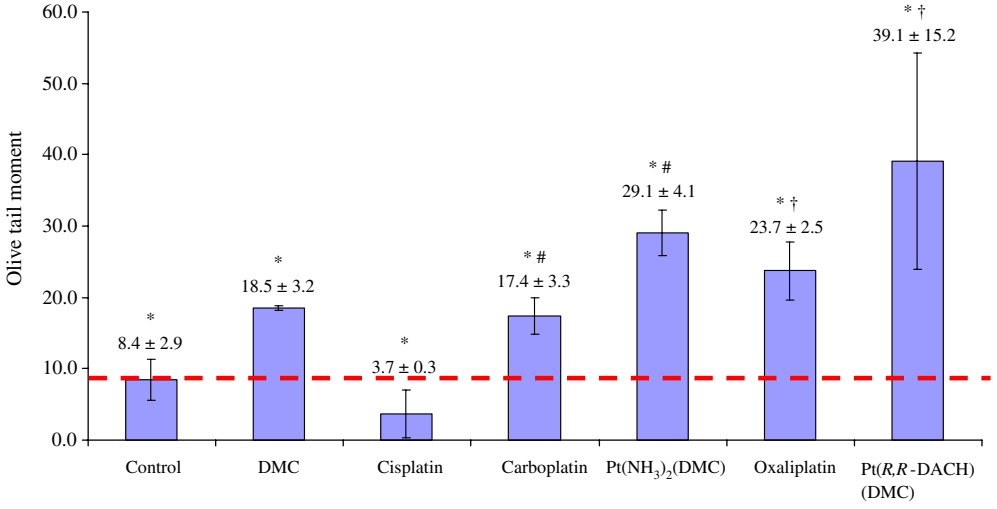

Fig. 3. The Olive tail moment ± standard deviation ($n = 3$) in HCT116 cells treated with 50 μM drugs for 3 h. $^*P < 0.05$ for drugs against the control; $^\#P < 0.05$ for $Pt(NH_3)_2$(DMC) against carboplatin; $^\dagger P < 0.05$ for Pt(*R,R*-DACH)(DMC) against oxaliplatin.

Table 1
Pt–DNA adduct levels ± standard deviation in HCT116 cells treated with 50 μM drugs for 3 h

Treatment	Pt–DNA adduct levels (pg of Pt/μg of DNA)
Cisplatin	26.7 ± 9.3[a]
Carboplatin	0.8 ± 0.4[a,b]
$Pt(NH_3)_2$(DMC)	3.0 ± 0.9[b]
Oxaliplatin	12.9 ± 4.8[a,c]
Pt(*R,R*-DACH)(DMC)	11.6 ± 3.7[c]

The number of experiments performed is 3–6.
[a] $P < 0.05$ for all treatments against the control.
[b] $P < 0.05$ for $Pt(NH_3)_2$(DMC) against carboplatin.
[c] $P > 0.05$ for Pt(R,R-DACH)(DMC) against oxaliplatin.

Pt(*R,R*-DACH)(DMC) > $Pt(NH_3)_2$(DMC) > oxaliplatin > DMC > carboplatin > carboplatin > control > cisplatin.

Formation of Pt–DNA adducts

GFAA determines the total amount of platinum–DNA adducts formed due to intra- and interstrand cross-links after drug treatment. The levels of Pt–DNA adduct formed for each tested compound are shown in Table 1. Cisplatin induced the highest level of Pt–DNA adducts, and carboplatin the lowest (range from 26.7 to 0.8 pg of Pt/μg of DNA). The amount of cisplatin–DNA adducts was approximately twice that formed with oxaliplatin (26.7 *vs.* 12.9 pg of Pt/μg of DNA). This result is consistent with previous literature reports that cisplatin forms 2–7 times more lesions than oxaliplatin [2,6]. When comparing the structurally similar $Pt(NH_3)_2$(DMC) and carboplatin, the results showed that the Pt–DNA adduct level for the former was higher (3.0 *vs.* 0.8 pg of Pt/μg of DNA, respectively; $P < 0.05$). Pt(*R,R*-DACH)(DMC) and oxaliplatin have the same platinum moiety, but when their levels of Pt–DNA adducts were compared, no significant difference was observed (11.6 *vs.* 12.9 pg of Pt/μg of DNA, respectively; $P > 0.05$). Interestingly, Pt(*R,R*-DACH)(DMC) also formed fewer Pt–DNA adducts than cisplatin (11.6 *vs.* 26.7 pg of Pt/μg of DNA, respectively; $P < 0.05$).

Assessment of sub-G_1 population by flow cytometry

In an attempt to determine whether the presence of DNA fragments as indicated by the alkaline comet assay was a result of apoptosis and/or necrosis, the sub-G_1 population was measured using flow cytometry. No significantly high sub-G_1 populations were found for any of the drug treatments when compared with the control (control: 0.91 ± 0.27%; drug treatments: 0.41–1.00%).

Discussion

The alkaline comet assay shows the net effect of platinum drug-induced DNA lesions resulting from the formation of inter- and intrastrand cross-links, as measured by OTMs. It has been suggested from various literature reports, that DNA migration is *retarded* by interstrand DNA cross-links [14,18,19], but *facilitated* by intrastrand cross-links. The latter effect is probably due to single strand breaks resulting from alkylated bases that are undergoing DNA repair [19], and that these bases are alkali-labile [20]. The general understanding is that 5–10% *interstrand* cross-links are formed with cisplatin treatment [3]; whereas for carboplatin, *intrastrand* cross-links apparently predominate, and 3–4% interstrand cross-links are detected only at very high concentrations of the drug (e.g. cells exposed to 700 μM for 12 h) [4]. Analogous to carboplatin, oxaliplatin induces either significantly low or negligible levels of interstrand cross-links [2,6].

In this study, treatment with cisplatin resulted in a reduced OTM relative to the control (Fig. 3), concurring with the report by Wozniak et al. [18]. The data suggest that a significant level of cisplatin-induced interstrand DNA cross-links has been generated, as demonstrated by the retardation of DNA migration during the electrophoresis step of the alkaline comet assay [14,18,19]. In contrast, treatment with oxaliplatin and carboplatin produced increased OTMs when compared with the control (Fig. 3), which is also consistent with literature findings [21].

The relatively low carboplatin concentrations used in this study (50 μM, 3 h) apparently limited the degree of interstrand DNA cross-links formed, when compared to cisplatin. The slow dissociation of the cyclobutyldicarboxylate ligand (CBDCA) from carboplatin may explain why fewer interstrand DNA cross-links are formed. With regards to oxaliplatin, it is highly probable that the steric bulk of the DACH moiety hindered the formation of interstrand DNA cross-links. A summary of the correlation between inter- and intrastrand DNA cross-links with OTM, illustrating the characteristically different behavior of cisplatin, oxaliplatin, and carboplatin, is depicted in Fig. 4.

The tail moment due to DMC treatment was increased relative to the control (Fig. 3), and it is apparent that DMC can cause DNA damage. This concurs with a similar finding that cantharidin could induce DNA damage as shown by a comet assay [12]; however, the damage causing mechanism remains unclear. It has been proposed that cantharidin may increase the oxidative stress in cells that subsequently lead to DNA damage [12]. Studies to date have provided some indirect evidence for a possible relationship between DNA damage induced by oxidative stress, and the cytotoxicity caused by cantharidin and DMC-like derivatives.

An earlier study conducted by Kok et al. using a DNA precipitation assay, reported a significant DNA strand break in KB cells (oral cancer) after treatment with DMC [22]. This DNA strand break is likely due to an apoptotic event rather than DNA lesions caused by DMC. This argument is supported by other findings that a significant sub-G_1 population and a DNA ladder were observed when colon cancer cells [11], oral cancer cells [23], and human

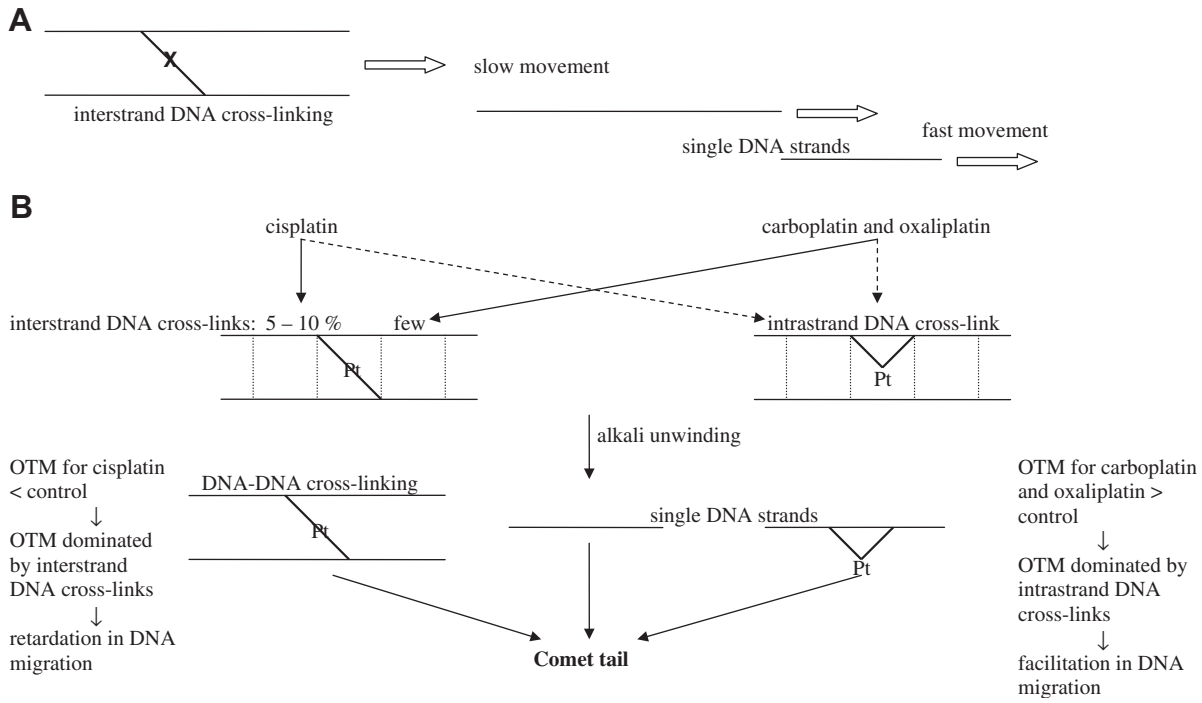

Fig. 4. Illustration of DNA migration under the electrophoresis step of an alkaline comet assay (A) for interstrand DNA cross-links and single DNA strand; (B) correlation of inter- (→) and intra- (⇢) strand DNA cross-links induced by platinum drugs with OTM.

leukemia cells [24] were treated with DMC under similar conditions. In this current study, flow cytometry results did not show a significantly high sub-G_1 population for all drugs when compared with the control. Thus, it appears that the results of the alkaline comet assay were due mainly to DNA lesions generated by different drugs without apoptotic and/or necrotic influence. This study is the first to demonstrate the use of a comet assay to establish DMC-induced DNA damage in HCT 116 cells.

Due to the structural similarity between $Pt(NH_3)_2$(DMC) and carboplatin, and that of Pt(*R,R*-DACH)(DMC) and oxaliplatin, their OTMs were compared. The results indicated that there was no significant difference in the level of Pt–DNA adducts formed between Pt(*R,R*-DACH)(DMC) and oxaliplatin (Table 1); but the tail moment for Pt(*R,R*-DACH)(DMC) was increased (Fig. 3), which can be attributed to the DMC ligand being released from Pt(*R,R*-DACH)(DMC) that induced additional DNA lesions. A similar analogy can be made for the $Pt(NH_3)_2$(DMC) complex where the level of Pt–DNA adducts was high with respect to carboplatin (Table 1). An increased tail moment for $Pt(NH_3)_2$(DMC) can be partly attributed to DNA damage induced by the released DMC ligand, and to the high level of Pt–DNA adducts formed.

Previously, we have shown by flow cytometry that $Pt(NH_3)_2$(DMC) was able to trigger an early onset of apoptosis when compared with carboplatin, and that it was more potent [11]. This observation can now be explained by the likelihood of more serious DNA damage caused by $Pt(NH_3)_2$(DMC) relative to carboplatin; arising in part from more Pt–DNA adducts being formed, and the released DMC inducing DNA damage.

Although Pt(*R,R*-DACH)(DMC) induced more DNA lesions than oxaliplatin, the cytotoxicity of the two compounds is similar [11,25,26], as were their cell cycle distribution patterns [11]. Therefore it can be deduced that in this case, the common *R,R*-DACH–Pt moiety in the two compounds apparently dictated cytotoxicity.

Furthermore, the results showed that the levels of Pt–DNA adduct formed for the DACH-containing Pt drugs were lower than for cisplatin, but in fact, oxaliplatin and Pt(*R,R*-DACH)(DMC) were more potent [11,25]. Hence it is reasonable to conclude that platinum compounds containing the *R,R*-DACH moiety are more effective antiproliferative agents.

In summary, the alkaline comet assay has enabled the correlation of the degree of intrastrand and interstrand cross-links formed in Pt-treated HCT116 cells based on OTM measurements; and allowed the demonstration of the characteristically different DNA-damaging behavior of the established Pt anticancer drugs and novel DMC–Pt complexes. From the results, it was also apparent that the DMC ligand released from Pt(*R,R*-DACH)(DMC) and $Pt(NH_3)_2$(DMC) complexes caused additional DNA lesions when compared with oxaliplatin and carboplatin, which would explain the superior antiproliferative effect of DMC-integrated Pt compounds.

This study has further highlighted that the innovative integration of DACH and DMC ligands with a Pt-moiety

resulted in highly potent anticancer agents; and that the mechanism of action is distinctly different from that of cisplatin and carboplatin.

Acknowledgments

We gratefully acknowledge the Research Grant Council of Hong Kong SAR for financial support (RGC Ref: 4357/04M). Technical assistance from Mr. Freddie Kwok, Department of Biology, and Ms. Jenny Hau, Department of Anatomy, The Chinese University of Hong Kong are acknowledged.

References

[1] E. Wong, C.M. Giandomenico, Current status of platinum-based antitumor drugs, Chem. Rev. 99 (1999) 2451–2466.
[2] E. Raymond, S. Faivre, S. Chaney, J. Woynarowski, E. Cvitkovic, Cellular and molecular pharmacology of oxaliplatin, Mol. Cancer Ther. 1 (2002) 227–235.
[3] S.R. Rajski, R.M. Williams, DNA cross-linking agents as antitumor drugs, Chem. Rev. 98 (1998) 2723–2795.
[4] F.A. Blommaert, H.C.M. Van Dijk-Knijmemburg, F.J. Dijt, L. den Engelse, R.A. Baan, F. Berends, A.M.J. Fichtinger-Schepman, Formation of DNA adducts by the anticancer drug carboplatin: different nucleotide sequence preferences in vitro and in cells, Biochemistry 34 (1995) 8474–8480.
[5] L. Stojic, R. Brun, J. Jiricny, Mismatch repair and DNA damage signaling, DNA Repair 3 (2004) 1091–1101.
[6] J.M. Woynarowski, W.G. Chapman, C. Napier, M.C.S. Herzig, P. Juniewicz, Sequence- and region-specificity of oxaliplatin adducts in naked and cellular DNA, Mol. Pharmacol. 54 (1998) 770–777.
[7] V. Brabec, J. Kasparkova, Modifications of DNA by platinum complexes: relation to resistance of tumors to platinum antitumor drugs, Drug Resist. Updat. 8 (2005) 131–146.
[8] M. Wei, S.M. Cohen, A.P. Silverman, S.J. Lippard, Effects of spectator ligands on the specific recognition of intrastrand platinum–DNA cross-links by high mobility group box and TATA-binding proteins, J. Biol. Chem. 276 (2001) 38774–38780.
[9] F. Massicot, H. Dutertre-Catella, C. Pham-Huy, X.-H. Liu, H.T. Due, J.-M. Warnet, In vitro assessment of renal toxicity and inflammatory events of two protein phosphatase inhibitors cantharidin and nor-cantharidin, Basic Clin. Pharmacol. 96 (2005) 26–32.
[10] A. McCluskey, A.T.R. Sim, J.A. Sakoff, Serine–threonine protein phosphatase inhibitors: development of potential therapeutic strategies, J. Med. Chem. 45 (2002) 1151–1175.
[11] C.-W. Yu, K.K.W. Li, S.-K. Pang, S.C.F. Au-Yeung, Y.-P. Ho, Anticancer activity of a series of platinum complexes integrating demethylcantharidin with isomers of 1,2-diaminocyclohexane, Bioorg. Med. Chem. Lett. 16 (2006) 1686–1691.
[12] T. Efferth, R. Rauh, S. Kahl, M. Tomicic, H. Böchzelt, M.E. Tome, M.M. Briehl, R. Bauer, B. Kaina, Molecular modes of action of cantharidin in tumor cells, Biochem. Pharmacol. 69 (2005) 811–818.
[13] S.C.F. Au-Yeung, P.S.K. Pang, Y.P. Ho, Innovative platinum derived anticancer agents-risk or opportunity? Nat. Rev. Drug Discov. 5 (2006) 5–9, doi:10.1038/nrd1691-c1.
[14] R.R. Tice, E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.-C. Ryu, Y.F. Sasaki, Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing, Environ. Mol. Mutagen. 35 (2000) 206–221.
[15] Z. Darzynkiewicz, G. Juan, DNA content measurement for dna ploidy and cell cycle analysis, in: J.P. Robinson, Z. Darzynkiewicz, J. Dobrucki, W.C. Hyun, J.P. Nolan, A. Orfao, Peter S. Rabinovitch (Eds.), Current Protocols in Cytometry, John Wiley and Sons Inc., 2003, Chapter 7, Unit 7.5.
[16] W.M. Strauss, Preparation of genomic dna from mammalian tissue, in: F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, Kevin Struhl (Eds.), Current Protocols in Molecular Biology, John Wiley & Sons, Inc., 2002, Chapter 2, Section I, Unit 2.2.
[17] G.J. Griffiths, M.Y. Koh, V.G. Brunton, C. Cawthorne, N.A. Reeves, M. Greaves, M.J. Tilby, D.G. Pearson, C.J. Ottley, P. Workman, M.C. Frame, C. Dive, Expression of kinase-defective mutants of c-Src in human metastatic colon cancer cells decreases Bcl-x_L and increases oxaliplatin- and Fas-induced apoptosis, J. Biol. Chem. 279 (2004) 46113–46121.
[18] K. Wozniak, A. Czechowska, J. Blasiak, Cisplatin-evoked fragmentation in normal and cancer cells and its modulation by free radical scavengers and tyrosine kinase inhibitor STI571, Chem. Biol. Interact. 147 (2004) 309–318.
[19] D.W. Fairbain, P.L. Olive, K.L. O'Neill, The comet assay: a comprehensive review, Mutat. Res. 339 (1995) 37–59.
[20] Y. Miyamae, K. Iwasaki, N. Kinae, S. Tsuda, M. Murakami, M. Tanaka, Y. Sasaki, Detection of DNA lesions induced by chemical mutagens using the single-cell gel electrophoresis (Comet) assay. Relationship between DNA migration and alkaline condition, Mutat. Res. 393 (1997) 107–113.
[21] K. Takagi, Y. Kawai, T. Yamauchi, T. Ueda, Inhibition of repair of carboplatin-induced DNA damage by 9-β-D-arabinofuranosyl-2-fluoroadenine in quiescent human lymphocytes, Biochem. Pharmacol. 68 (2004) 1757–1766.
[22] S.H. Kok, C.Y. Hong, M.Y.P. Kuo, C.H.K. Lee, J.J. Lee, I.U. Lou, M.S. Lee, M. Hsiao, S.K. Lin, Comparisons of norcantharidin cytotoxic effects on oral cancer cells and normal buccal keratinocytes, Oral Oncol. 39 (2003) 19–26.
[23] S.-H. Kok, S.-J. Cheng, C.-Y. Hong, J.-J. Lee, S.-K. Lin, Y.-S. Kuo, C.-P. Chiang, Mark Y.-P. Kuo, Norcantharidin-induced apoptosis in oral cancer cells is associated with an increase of proapoptotic to antiapoptotic protein ratio, Cancer Lett. 217 (2005) 43–52.
[24] J.-L. Li, Y.-C. Cai, X.-H. Liu, L.-J. Xian, Norcantharidin inhibits DNA replication and induces apoptosis with the cleavage of initiation protein Cdc6 in HL-60 cells, Anticancer Drug 17 (2006) 307–314.
[25] J. Boyer, E.G. McLean, S. Aroori, P. Wilson, A. McCulla, P.D. Carey, D.B. Longley, P.G. Johnston, Characterization of p53 wild-type and null isogenic colorectal cancer cell lines resistant to 5-fluorouracil, oxaliplatin, and irinotecan, Clin. Cancer Res. 10 (2004) 2158–2167.
[26] T.V. Rakitina, I.A. Vasilevskaya, P.J. O'Dwyer, Additive interaction of oxaliplatin and 17-allylamino-17-demethoxygeldanamycin in colon cancer cell lines results from inhibition of nuclear factor κB signaling, Cancer Res. 63 (2003) 8600–8605.