Bioorganic & Medicinal Chemistry Letters 20 (2010) 2448–2451

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Towards biomarker-dependent individualized chemotherapy: Exploring cell-specific differences in oxaliplatin–DNA adduct distribution using accelerator mass spectrometry

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ARTICLE INFO

Article history:
Received 18 December 2009
Revised 2 March 2010
Accepted 4 March 2010
Available online 7 March 2010

Keywords:
Oxaliplatin
DNA adduct
Accelerator mass spectrometry

ABSTRACT

Oxaliplatin is a third-generation platinum-based anticancer drug that is currently used in the treatment of metastatic colorectal cancer. Oxaliplatin, like other platinum-based anticancer drugs such as cisplatin and carboplatin, is known to induce apoptosis in tumor cells by binding to nuclear DNA, forming monoadducts, and intra- and interstrand diadducts. Previously, we reported an accelerator mass spectrometry (AMS) assay to measure the kinetics of oxaliplatin-induced DNA damage and repair (Hah, S. S.; Sumbad, R. A.; de Vere White, R. W.; Turteltaub, K. W.; Henderson, P. T. *Chem. Res. Toxicol.* **2007**, *20*, 1745). Here, we describe another application of AMS to the measurement of oxaliplatin–DNA adduct distribution in cultured platinum-sensitive testicular (833 K) and platinum-resistant breast (MDA-MB-231) cancer cells, which resulted in elucidation of cell-dependent differentiation of oxaliplatin–DNA adduct formation, implying that differential adduction and/or accumulation of the drug in cellular DNA may be responsible for the sensitivity of cancer cells to platinum treatment. Ultimately, we hope to use this method to measure the intrinsic platinated DNA adduct repair capacity in cancer patients for use as a biomarker for diagnostics or a predictor of patient outcome.

(*trans-R,R*)1,2-Diaminocyclohexaneoxalatoplatinum(II) (oxaliplatin) derives from the well-established platinum drug *cis*-diamminedichloroplatinum(II) (cisplatin) (structures shown in Fig. 1). It is the first platinum-based anticancer drug approved for the treatment of colorectal cancer, which is a major cause of cancer-related death in developed countries and is essentially equally distributed between men and women.[1] Oxaliplatin has been licensed in the European Union since 1999, and in the United States since 2002.[2] Oxaliplatin has a broad spectrum of anticancer activity, and importantly, preclinical studies showed that oxaliplatin in combination with 5-FU has greater in vitro and in vivo antiproliferative activity than either compound alone in several tumor models, including colon cancer.[3]

Oxaliplatin has diaminocyclohexane (DACH) as a carrier group and oxalato as a leaving group, and is known to exert the cytotoxicity effect by interaction with DNA to form monoadducts and intra- and interstrand diadducts (Fig. 2), like other platinum-based anticancer drugs such as cisplatin and *cis*-diammine(1,1-cyclobutyldicarboxylato)platinum(II) (carboplatin).[3] Importantly, oxaliplatin shows a better safety profile than cisplatin in clinical studies, except for reversible damage to peripheral sensory nerves,[2] and a lack of cross-resistance with cisplatin or carboplatin, presumably because DACH–Pt–DNA adducts formed by oxaliplatin are bulkier and more hydrophobic than cisplatin and carboplatin adducts, leading to different effects in cells.[4] However, the clinical efficacy and toxicity resulting from oxaliplatin treatment vary from patient to patient, largely because of intrinsic and acquired resistance.[2] Although resistance remains poorly understood, it is clearly influenced by variation in cellular rates of drug influx, export, and the formation and repair of platinum DNA adducts.

In spite of extensive analytical studies for Pt–DNA adduct formation/distribution under pharmacologically- or subpharmacologically-relevant conditions,[3] measurement of the adducts in cells and in vivo with higher sensitivity and precision is still desirable, because it remains unclear which of the Pt–DNA adducts are responsible for the cytotoxic activity of platinum-based anticancer drugs. To address this issue, a sensitive and quantitative assay would be ideal to better understand mechanisms of resistance and to be predictive of patient outcome.

We recently reported the use of an extremely sensitive detection method of accelerator mass spectrometry (AMS),[5] a highly sensitive technique with origins in geochemistry for radiocarbon dating, in order to study the kinetics of oxaliplatin–DNA adduct formation in DNA, both in vitro and in cultured cells, as well as

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doi:10.1016/j.bmcl.2010.03.020

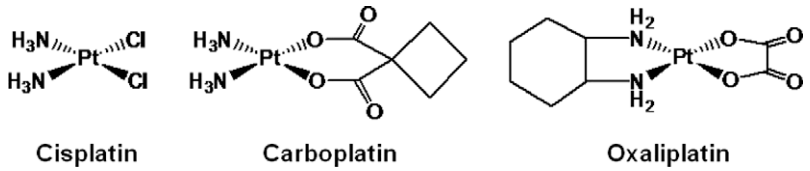

Figure 1. Chemical structures of cisplatin, carboplatin, and oxaliplatin.

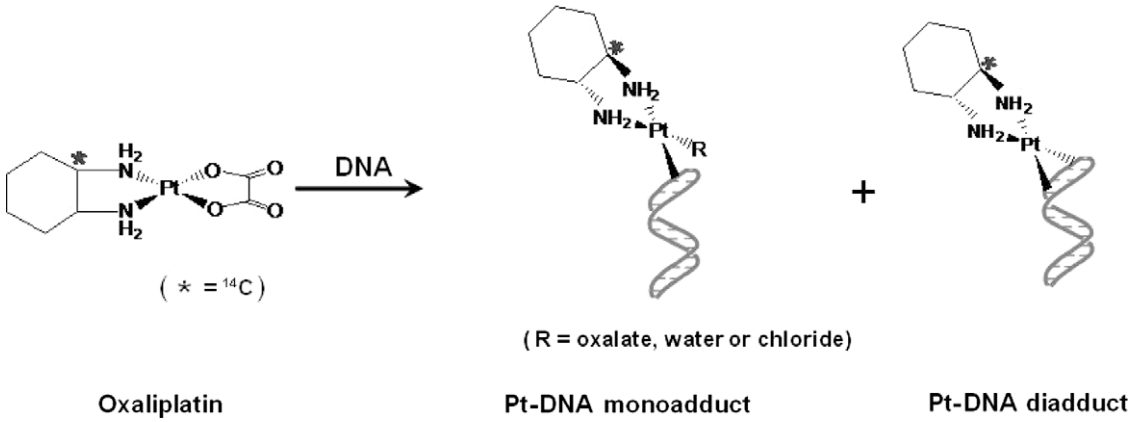

Figure 2. Simplified scheme for the reaction of oxaliplatin with DNA (the location of the ^{14}C atom is asterisked). Because radiocarbon is located in the DACH carrier group, radiocarbon in oxaliplatin–DNA monoadducts and diadducts can be detected by AMS.

to study rates of cellular drug influx, efflux, DNA damage and DNA repair in cultured platinum-sensitive testicular (833 K) and platinum resistant breast and bladder (MDA-MB-231 and T24, respectively) cancer cells incubated with a subpharmacological dose of oxaliplatin (0.2 μM) containing 2.16 pmol of [^{14}C]oxaliplatin.[6] Like other experiments using radioisotopes, AMS is specific only to the labeled compound in any chemical or biological medium, allowing the direct determination of the amount of isotope in a sample and thus the quantitative analysis of the fate of the radiolabeled probes under the given conditions.[5] The lowest concentration of radiocarbon measured in our previous work was approximately 1 ± 0.1 amol/μg of DNA, when assaying 1 μg of DNA, of which the sensitivity for measuring oxaliplatin–DNA adducts is still the highest reported to date.[6]

In the present study, we report an application of our previous approach to those of oxaliplatin–DNA adduct distribution in cultured 833 K and MDA-MB-231 cancer cell lines of differing platinum sensitivity, so as to investigate oxaliplatin–DNA adduct formation and/or accumulation in these cells compared to that in T24 cells. We applied the same experimental conditions as reported in our previous oxaliplatin study,[6,7] and determined oxaliplatin–DNA adduct distribution in 833 K and MDA-MB-231 cells which were incubated with a subpharmacological concentration of oxaliplatin (0.2 μM) for 24 h, respectively. This is a clinically relevant concentration for this assay, since plasma levels of oxaliplatin are reported to be a maximum of ~3.6 μM in patients undergoing therapy.[8] This drug concentration allowed measurement of the accumulation of radiocarbon in cells and DNA without interference from acute toxicity.[6]

We were able to take advantage of radiocarbon located in the DACH carrier group. By following the DNA extraction, digestion and HPLC separation conditions in the literature,[6,9] oxaliplatin–DNA adducts were characterized at the nucleoside level for mono- and diadduct formation using AMS because those adducts still have radiocarbon (Fig. 2). Genomic DNA extracted from 833 K and MDA-MB-231 cells was enzymatically digested by deoxyribonuclease and P1 nuclease to deoxyribonucleotides, and the resulting mixture was filtered and separated by HPLC. Fractions were taken at one minute intervals and then dried and converted to graphite for AMS measurement.

Figure 3 shows the retention times of the resulting radiocarbon-containing fractions, as compared to UV detection of authentic standards of the Pt(DACH)-adducted nucleotides and nucleosides which were prepared according to the literature.[6,9] The resulting distribution of peaks could be assigned to several types of Pt(DACH) mono- and diadducts. The obtained data clearly show the presence of intrastrand and interstrand DNA adducts in addition to at least two products that were not observed in the previously shown experiments with naked DNA. The total radiocarbon contents in extracted DNA in MDA-MB-231 and 833 K cells were determined to be 3.1 ± 0.6 and 6.3 ± 0.4 amol ^{14}C/μg of DNA, respectively, as previously reported,[6] suggesting that approximately 1.2% of the cellular radiocarbon was found in nuclear DNA after one-day incubation, with the highest accumulation in the platinum-sensitive 833 K cells, which may be explained by DNA repair deficiencies in these cells as compared to the other cell types. The total radiocarbon recovered by HPLC relative to the undigested control was approximately 98% (Table 1). Because we had essentially quantitative recovery of the total injected radiocarbon from each HPLC injection, the data were indicative of the total DNA adduct distribution in the cellular DNA for the particular experimental conditions used in this study. Our observation also confirms previous Letters that there is a high correlation between decreased adduct accumulation and resistance to platinum.[10] Interestingly, the mass distribution of the oxaliplatin–DNA adducts in MDA-MB-231 cells is also different from that of T24 cells. These results support that different cancer cell types may have contrasting abilities to repair DNA damage and tolerate platinum chemotherapy.

From a quantitative point of view, our data for oxaliplatin-adduct distribution in MDA-MB-231 cells suggest that after a 24-h incubation, monoadducts constituted approximately 6% of the total radioactivity from the HPLC fractions and oxaliplatin forms approximately 4% intrastrand Pt-1,2-d(GpG), 3% intrastrand Pt-