Quantitative methods for studying DNA interactions with chemotherapeutic cisplatin

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Platinum-containing anti-cancer drugs (e.g., cisplatin) exert their biological effects by forming DNA adducts, so highly sensitive, specific quantitative methods are needed to correlate the molecular dose of these adducts with the effects of treatment. DNA adducts may also give information with regard to understanding drug resistance. In this work, we briefly review and compare existing quantitative strategies for the determination of the most important platinum-DNA adducts (e.g., immunochemical assays, ^{32}P-post-labeling and mass spectrometry-based methods). We examine the advantages and the disadvantages of the different strategies. In addition, we report important information on the analytical figures of merit of the different methodologies and the problems associated with sample processing.

Keywords: Antibody; Cancer; Cisplatin; DNA adduct; Immunochemical assay; Inductively coupled plasma; ICP-MS; Mass spectrometry; Post-labeling, Quantification; Sample processing

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1. Introduction

Cis-diamminedichloroplatinum (II) (cisplatin or cDDP) is the most widely-used cancer chemotherapeutic metallodrug. Cisplatin-based chemotherapy displays significant anti-tumor activity against cancers of testis, ovary, head, neck and lung [1,2]. This biological activity of cisplatin and other platinum-containing anti-cancer compounds is based upon the formation of covalently-linked products (adducts) with nucleophilic sites in the nucleic-acid structure that block DNA replication and transcription and, ultimately, cell division that finally leads to cell death [3–5]. The most abundant adducts are the intrastrand cross-links between two adjacent purine bases, guanine-guanine (Pt-GG, 65%) or adenine–guanine (Pt-AG, 25%), as can be seen in Fig. 1 [6–9]. These adducts could be used as biomarkers in order to provide quantitative data concerning the molecular dose and biological effects of platinum-based chemotherapy. Moreover, they could also have a predictive value with respect to clinical response to such treatments.

Unfortunately, the clinical use of Pt-based drugs, especially cisplatin, in chemotherapy is hampered by severe side effects, mainly ototoxicity and nephrotoxicity [10,11] as well as intrinsic, acquired resistance to the drug [5,12,13]. Although this resistance remains poorly understood, it is clearly related to the extension of DNA damage, so, to address the formation of cisplatin-DNA adducts in biological samples quantitatively could offer powerful new insights in better understanding molecular resistance to Pt-based drugs. For this purpose, both high analytical sensitivity and high selectivity are required, because the content of adducts in DNA is very small compared with those of normal bases, and, generally, only small amounts of DNA samples are available for analysis. Over the past 20 years, there has been a gradual improvement in the analytical approaches used to study DNA adducts, both qualitatively and quantitatively. However, there is still a lack of sensitive, accurate and robust strategies to permit quantitative determination of DNA adducts with Pt-based drugs, specifically cisplatin.

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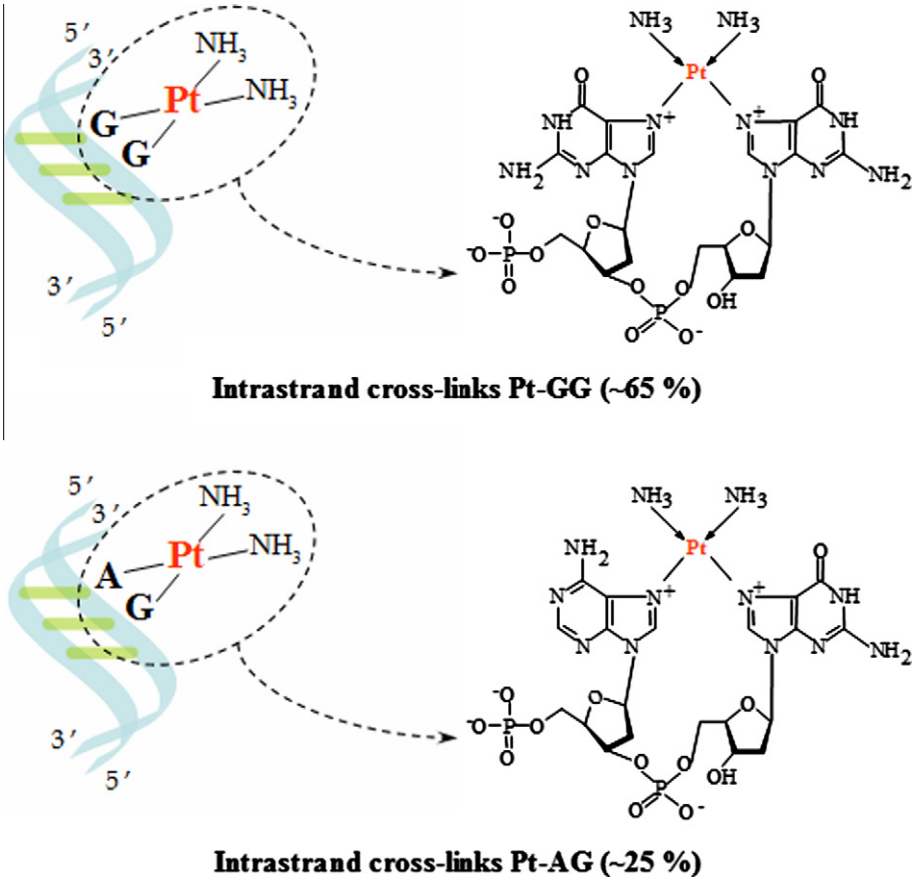

Figure 1. Structure of the main bidentate cisplatin-DNA adducts: Pt-GG and Pt-AG.

Among the analytical approaches currently used for measuring DNA adducts, we can include ^{32}P-post-labeling, immunoassay using antibodies against modified DNA, conventional mass spectrometry (MS) and accelerator MS [14]. These techniques permit detection of adducts *in vivo* in the range from 1 adduct per 10^{11} nucleotides (as found in humans at background levels or after low-level exposure to genotoxic compounds) to 1 adduct per 10^4 nucleotides (as found in animals treated with carcinogenic doses of chemicals) [14]. It may be difficult to further improve the sensitivity of some of the techniques used for adduct determination because of physico-chemical or biological limitations (e.g. the maximum available specific activity of [γ-^{32}P]-ATP, used in the post-labeling assay, or the highest achievable antibody-binding affinities for immunoassays). MS sensitivity is enhanced year by year, as the efficiency of procedures for ionization, transmission and detection of ions is continually improved. However, we should point out that the commercial availability of the labeled species used as internal standards for such accurate quantifications can be a major limitation.

This review brings together all the reported methods for quantifying DNA adducts, in relation to Pt-containing drugs currently used and critically compares the advantages and the limitations of each method, including potentially powerful emerging tools [e.g., inductively coupled plasma MS (ICP-MS)-based methods].

2. Analytical approaches

2.1. Immunochemical assays

Several immunochemical techniques have been developed to quantify DNA-platination products through the utilization of polyclonal or monoclonal antibodies in standard procedures [e.g., radioimmunoassay and, mainly, enzyme-linked immunosorbent assay (ELISA)]. Some of these antibodies are raised against platinum adducts in intact DNA. However, ascertaining the exact specificity of an antibody raised against modified polymeric DNA is difficult because the immmunogen generally carries a variety of DNA modifications. This problem is avoided by using antibodies specific to the different platinum adducts in digested DNA after chromatographic fractionation of the digest. Recognition of platinum adducts using polyclonal and monoclonal antibodies at the cellular level by immunohistochemistry analysis has also been shown to be possible [15].