

ORIGINAL ARTICLE

Genotoxicity of cisplatin and carboplatin in cultured human lymphocytes: a comparative study

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ABSTRACT

Cisplatin and carboplatin are integral parts of many antineoplastic management regimens. Both platinum analogues are potent DNA alkylating agents that robustly induce genomic instability and promote apoptosis in tumor cells. Although the mechanism of action of both drugs is similar, cisplatin appears to be more cytotoxic. In this study, the genotoxic potential of cisplatin and carboplatin was compared using chromosomal aberrations (CAs) and sister-chromatid exchange (SCE) assays in cultured human lymphocytes. Results showed that cisplatin and carboplatin induced a significant increase in CAs and SCEs compared to the control group ($p < 0.01$). Levels of induced CAs were similar in both drugs; however, the magnitude of SCEs induced by cisplatin was significantly higher than that induced by carboplatin ($p < 0.01$). With respect to the mitotic and proliferative indices, both cisplatin and carboplatin significantly decreased mitotic index ($p < 0.01$) without affecting the proliferative index ($p > 0.05$). In conclusion, cisplatin was found to be more genotoxic than carboplatin in the SCE assay in cultured human lymphocytes, and that might explain the higher cytotoxicity of cisplatin.

KEY WORDS: cisplatin; carboplatin; DNA damage; chromosomal aberrations; sister-chromatid exchange

Introduction

Cisplatin is a widely used antineoplastic drug that belongs to the DNA alkylating family (Rjiba-Touati *et al.*, 2013). It binds to nucleophilic groups in DNA, introducing intrastrand and interstrand DNA cross-links which lead to growth inhibition and apoptosis. Cisplatin is first line in the treatment of several types of cancer, including ovarian, lung, head and neck, CNS, endometrial, esophageal, bladder, breast, and cervical cancers, as well as osteogenic sarcoma and neuroblastoma (de Castria *et al.*, 2013; Aguiar *et al.*, 2016; Castrellon *et al.*, 2017).

Carboplatin is a similar organoplatinum that possesses DNA alkylating properties and interferes with DNA duplication in a similar fashion to cisplatin (Bruning & Mylonas, 2011). Carboplatin is currently implicated in

the treatment of several types of cancers, most of which overlap with those of cisplatin (Duan *et al.*, 2016; Fennell *et al.*, 2016). Carboplatin is also used in preparation for a stem cell or bone marrow transplantation (Agarwala *et al.*, 2011). Chemically, the two drugs are different in that carboplatin has a bidentate dicarboxylate in place of the two chloride ligand groups of cisplatin (Kralovanszky *et al.*, 1988).

While the two compounds share great similarity, cisplatin and carboplatin have a slightly different pharmacology. Carboplatin is relatively more stable inside the cells and is cleared more rapidly from the body (Duffull & Robinson, 1997). However, despite the lower toxicity profile, carboplatin can still cause myelosuppression which leads to neutropenia and consequently severe infections by opportunistic microbes (Pastor *et al.*, 2015).

More importantly, meta-analyses have shown a slight advantage of cisplatin-based therapy (Hotta *et al.*, 2004; Ardizzoni *et al.*, 2007) suggesting a different tumoricidal profile between the two drugs. Both drugs can equally induce platinum-DNA adducts but at different

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aquation rates (Hongo *et al.*, 1994). Furthermore, both drugs strongly activate components of the DNA damage response; however, phosphorylation of Chk1, H2AX and RPA2 is induced earlier by cisplatin than by carboplatin (Cruet-Hennequart *et al.*, 2009). Therefore, the aim of the current study is to further examine possible differences of the genotoxic effects of carboplatin and cisplatin at the chromosome level. Furthermore, the genotoxic effects of antineoplastic drugs in non-tumor cells are of special significance due to the risk of induction of secondary tumors in cancer patients.

To obtain relevant information, we utilized cultured human lymphocytes and sister-chromatid exchange (SCE) and chromosomal aberration (CA) assays. The SCE assay measures the exchange of genetic material between two identical sister chromatids and is greatly affected by mutagenic agents, while CA is used to measure chromosomal damage induced by clastogenic agents (Clare, 2012).

Material and methods

Blood donors

Five healthy subjects donated their blood for the study. Donors were recruited from the university campus using wall advertisements. All donors were non-smokers and non-alcoholic adults, aged 22–24 years. Donors had not been taking any medications or supplements for the past three months prior to blood donations. Ten milliliters of heparinized blood were collected from each donor in sterile vacutainer tubes. All donors gave written informed consents according to the Institutional Review Board prior to their participation in the study.

Drugs

Cisplatin (Ebewe Pharma) and carboplatin (Thymoorgan Pharmazie- Hikma) were dissolved in normal saline before beginning of the experiments. These drugs were initially in therapeutic concentrations and then they were serially diluted. The concentrations used in this study to evaluate the potential genotoxic side effects on normal lymphocytes were based on their known therapeutic half maximal inhibitory concentrations (IC₅₀) on leukemia cell lines (CEM, HL60, U937, K562). The concentrations were evaluated on normal leukocytes. Procedures for SCE and CA analysis, described later, were carried out. The microscopic slides were divided using horizontal lines 1 mm apart and the number of dead versus live cells in each line was counted. A ratio of approximately 50% was used as a cutoff to accept the concentrations used for the purpose of this study. The chosen final concentrations of the drugs in cell culture were the mean concentrations used on different leukemia cell lines and these were 0.4 µg/mL for cisplatin and 6.2 µg/mL for carboplatin (Wu-Chou Su *et al.*, 2000).

Cell cultures

Fresh blood was used to initiate lymphocytes cultures. About one mL of withdrawn blood was added

to tissue-culture flasks containing nine ml of media (Chromosome Medium P – EuroClone, Italy: RPMI 1640 medium supplemented with suitable amount of fetal bovine serum, glutamine, penicillin-streptomycin and phytohemagglutinin).

Sister-chromatid exchange assay

The procedure that was followed to conduct SCEs in cultured human lymphocytes was described previously (Khabour *et al.*, 2011). In brief, a fresh solution of Bromodeoxyuridine (BrdU, Sigma-Aldrich, final concentration 20 µg/ml) was added to cultured human lymphocytes directly after culture initiation. Cultures were then incubated at 37 °C in dark CO₂ incubator for 72 hours. Cisplatin/Carboplatin were added to cultures in the last 24 hours of incubation time (Al-Sweedan *et al.*, 2012). Before harvesting cultured lymphocytes, Colcemid (obtained from Euro clone, Italy, final concentration 10 µg/mL) was added to cultures for 90 minutes. Cultures were then centrifuged and the pellet was introduced to a hypotonic solution (0.075M KCl, Euro clone, Italy) and incubated at 37 °C for 20 min. Tubes were then centrifuged and the cellular pellet was fixed with three changes of ice-cold methanol:acetic acid (3:1, Carlo erba, China). Metaphase spreads were prepared on pre-chilled slides as previously described (Azab *et al.*, 2017). The slides were stained with the fluorescent-plus-Giemsa technique and SCEs were scored blindly using medical microscope at 1000× magnification. Fifty M2 metaphases were analyzed per each drug concentration/donor. In addition, M1, M3 and M4 metaphases were counted for analysis of mitotic and proliferative indices (Azab *et al.*, 2009; Alzoubi *et al.*, 2014).

Chromosomal aberrations (CAs) assay

CA assay cultures were prepared and treated similarly to those of SCE but without the addition of BrdU to the culture. After staining with 2% Giemsa solution (Medical Expertise House, Jordan, pH 6.8), 500 metaphase spreads (100 for each treatment/donor) were analyzed for CAs (Alzoubi *et al.*, 2012; Mhaidat *et al.*, 2016). Only breaks and exchanges were included in the analysis of CAs.

Cell kinetics analysis

The mitotic index and proliferative index were examined to reflect cytotoxicity of Cisplatin and Carboplatin. To determine the mitotic index for each concentration, at least 5,000 cells (1,000 cells from each donor) were included and the number of metaphases was counted. The mitotic index was calculated as the ratio of the number of metaphases seen vs. total number of intact cells seen. For the cell proliferation index the following calculation was used: $(M1 + 2 * M2 + 3 * M3) / 100$ for each slide, with a total of 500 metaphase cells used (Azab *et al.*, 2009).

Statistical analysis

Statistical comparisons were performed using GraphPad Prism statistical software (version 4) ANOVA, followed by Tukey *post hoc* test for analysis of the three

groups. A $p < 0.05$ was used as a threshold for statistical significance.

Results

Chromosomal breaks and exchanges were scored using metaphases stained with Giemsa. Chromosomal/chromatid breaks and exchanges were included in the CAs assessment. First, both cisplatin and carboplatin significantly increased CAs by 3.0 and 2.3 fold, respectively ($p < 0.01$, Figure 1). Interestingly, the level of the increase in CAs was slightly higher in the cisplatin-treated group than that in the carboplatin-treated group, despite lack of statistical significance ($p > 0.05$).

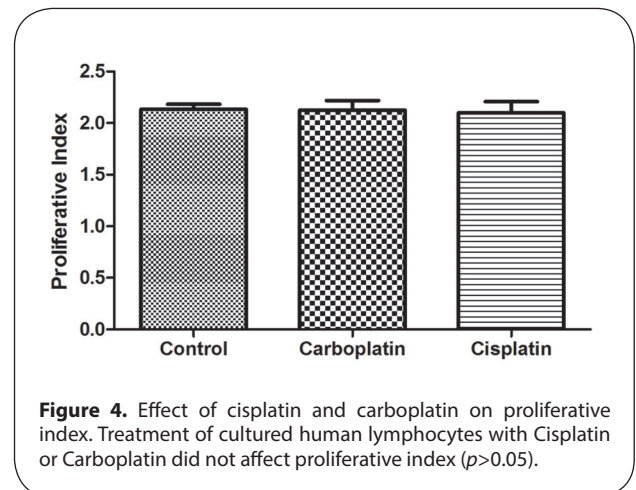
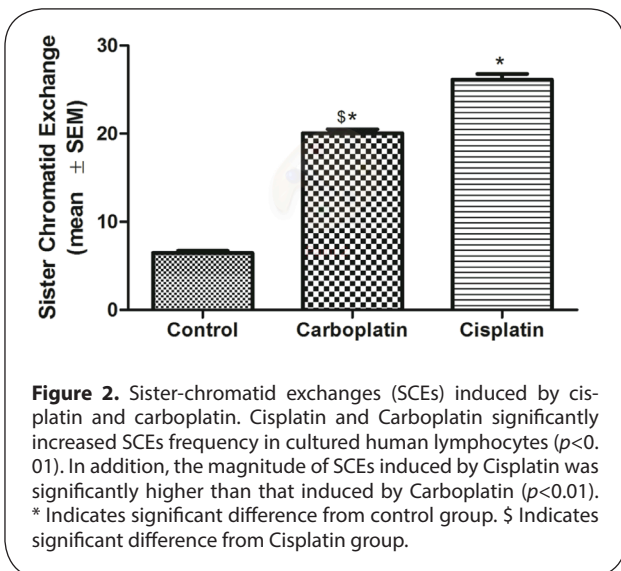
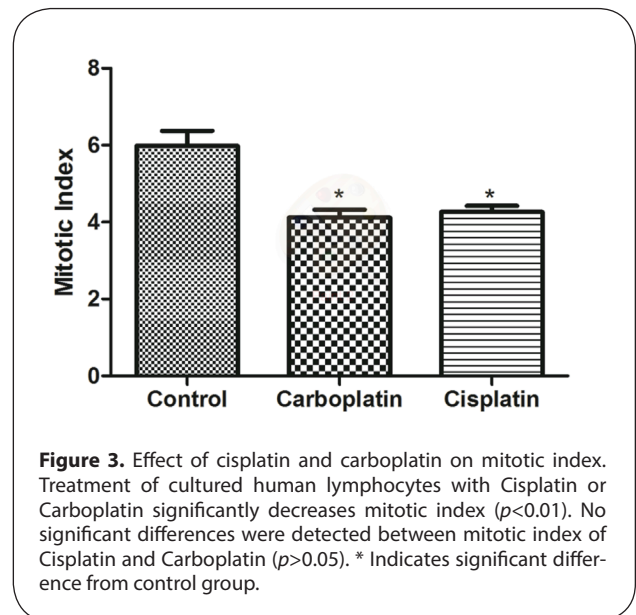
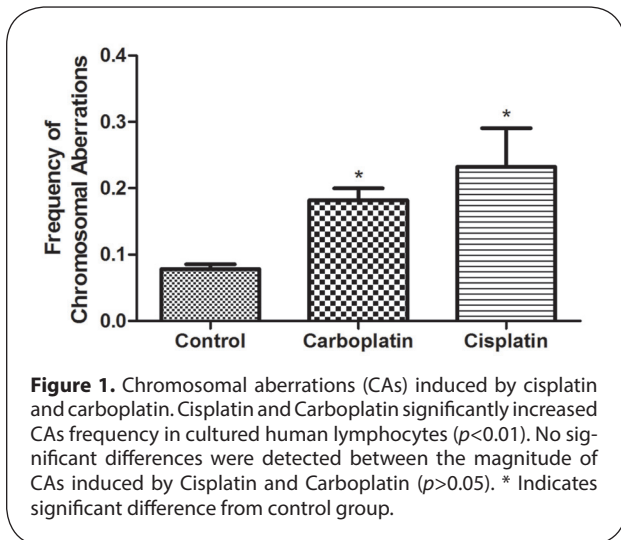
Figure 2 shows the frequency of SCEs induced by each treatment. Cisplatin and carboplatin significantly increased SCEs 4.05 and 3.1 fold, respectively ($p < 0.01$). The level of the increase in SCEs was significantly higher in the cisplatin-treated group than in the carboplatin-treated group ($p < 0.01$). Thus cisplatin induced more

genotoxicity in terms of SCEs than did carboplatin, a result that consistently agrees with previous observations (Shinkai *et al.*, 1988).

Finally, we wanted to examine if these genotoxic differences between cisplatin and carboplatin have variable outcomes on the proliferative potential of cultured cells. Both cisplatin and carboplatin significantly decreased the mitotic index ($p < 0.01$, Figure 3) without affecting the proliferative index ($p > 0.05$, Figure 4). However, mitotic and proliferative indices were similar in cisplatin and carboplatin groups ($p > 0.05$), indicating that the discrepancy in the genotoxic profiles of the two compounds is not necessarily responsible for the difference in their cytotoxic effect.

Discussion

In the current study, the genotoxicity of cisplatin and carboplatin was compared in cultured human lymphocytes. Results showed that cisplatin was more genotoxic



than carboplatin in the induction of SCEs. However, both drugs induced comparable levels of CAs, MI and PI.

The genotoxicity of cisplatin is well documented both *in vivo* and *in vitro* systems. This includes induction of SCEs, CAs, micronuclei and oxidative DNA damage in different models such as mice, rats, humans and cell lines (PC12, Ehrlich ascites tumor, HepG2) (Brozovic *et al.*, 2009; Desai & Gadhia, 2012; Ghosh *et al.*, 2013; Khabour *et al.*, 2014). Similarly, carboplatin was shown to induce chromosomal DNA damage in humans, animals and cell lines. For example, CAs and SCEs were shown to be induced by carboplatin in cultured human lymphocytes (Shinkai *et al.*, 1988), Ehrlich ascites tumor cells (Mylonaki-Charalambours *et al.*, 1998), and Chinese hamster ovarian cells (Gonzalez Cid *et al.*, 1995). The results that cisplatin is more potent in inducing SCEs is in agreement with a previous report conducted on Chinese hamster V 79 cells (Chibber & Ord, 1989). Thus the data presented in this study and those of other authors suggest that cisplatin is more genotoxic than carboplatin on normal cells. However, as previous works have shown, the genotoxic effects of cisplatin have not been correlated with increased incidence of cancer development in patients who received it. By extent, due to its lower genotoxic properties, carboplatin can be considered safe with regard to risk of secondary cancer development (Shinkai *et al.*, 1988).

The mechanisms for the induction of CAs and SCEs were suggested to be different. SCEs arise when damaged DNA induces the replicative bypass repair mechanisms during cell replication (Sasaki, 1980), whereas CAs are induced by damage repaired by post-replication repair processes. The differences in the ability of cisplatin and carboplatin in the induction of SCEs could be related to the type/magnitude of DNA damage they induced and how they interact with DNA. For example, carboplatin was shown to exhibit lower reactivity and slower DNA binding kinetics than cisplatin, although both form similar reaction products *in vitro* at equivalent doses (Hah *et al.*, 2006). With respect to treatment effectiveness, as noted earlier, it is well accepted that carboplatin has a relatively lower potency than cisplatin in treatment of some cancers (Moncharmont *et al.*, 2011). Therefore, all these mechanisms might contribute to the higher frequency of SCEs induced by cisplatin compared to carboplatin.

On the other hand, the comparable MI and PI can be attributed to the fact that the actual damage of the chromosomes measured by CA was similar between cisplatin and carboplatin. This means that the magnitude of damaged chromosomes in the two groups is comparable. Thus this translates to a comparable cytotoxic effect evaluated by PI and MI.

In the current investigation, only one dose of cisplatin and carboplatin were investigated and for one period. Future studies that examine a comprehensive concentration-effect of both drugs and for different periods are strongly recommended.

In conclusion, cisplatin was found to be more genotoxic than carboplatin in the SCE assay in cultured human lymphocytes.

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REFERENCES

- Agarwala AK, Perkins SM, Abonour R, Brames MJ, Einhorn LH. (2011). Salvage chemotherapy with high-dose carboplatin and etoposide with peripheral blood stem cell transplant in patients with relapsed pure seminoma. *Am J Clin Oncol* **34**: 286–288.
- Aguiar PN, Jr., Tadokoro H, da Silva GF, Landgraf MM, Noia Barreto CM, Filardi BA, Lopes GL, Jr., Oliveira P, de Mello RA. (2016). Definitive chemoradiotherapy for squamous head and neck cancer: cisplatin versus carboplatin? A meta-analysis. *Future Oncol* **12**: 2755–2764.
- Al-Sweedan SA, Khabour O, Isam R. (2012). Genotoxicity assessment in patients with thalassemia minor. *Mutat Res* **744**: 167–171.
- Alzoubi K, Khabour O, Hussain N, Al-Azzam S, Mhaidat N. (2012). Evaluation of vitamin B12 effects on DNA damage induced by pioglitazone. *Mutat Res* **748**: 48–51.
- Alzoubi K, Khabour O, Khader M, Mhaidat N, Al-Azzam S. (2014). Evaluation of vitamin B12 effects on DNA damage induced by paclitaxel. *Drug Chem Toxicol* **37**: 276–280.
- Ardizoni A, Boni L, Tiseo M, Fossella FV, Schiller JH, Paesmans M, Radosavljevic D, Paccagnella A, Zatloukal P, Mazzanti P, Bisset D, Rosell R. (2007). Cisplatin- versus carboplatin-based chemotherapy in first-line treatment of advanced non-small-cell lung cancer: an individual patient data meta-analysis. *J Natl Cancer Inst* **99**: 847–857.
- Azab M, Khabour OF, Al-Omari L, Alzoubi MA, Alzoubi K. (2009). Effect of every-other-day fasting on spontaneous chromosomal damage in rat's bone-marrow cells. *J Toxicol Environ Health A* **72**: 295–300.
- Azab M, Khabour OF, Alzoubi KH, Hawamdeh H, Quttina M, Nassar L. (2017). Assessment of genotoxicity of pyrethrin in cultured human lymphocytes. *Drug Chem Toxicol* **40**: 251–255.
- Brozovic G, Orsolich N, Knezevic F, Horvat Knezevic A, Benkovic V, Sakic K, Hrgovic Z, Bendelja K, Fassbender WJ. (2009). Genotoxicity and cytotoxicity of cisplatin treatment combined with anaesthetics on EAT cells *in vivo*. *Onkologie* **32**: 337–343.
- Bruning A, Mylonas I. (2011). New emerging drugs targeting the genomic integrity and replication machinery in ovarian cancer. *Arch Gynecol Obstet* **283**: 1087–1096.
- Castrellon AB, Pidhorecky I, Valero V, Raez LE. (2017). The Role of Carboplatin in the Neoadjuvant Chemotherapy Treatment of Triple Negative Breast Cancer. *Oncol Rev* **11**: 324.
- Clare G. (2012). The *in vitro* mammalian chromosome aberration test. *Methods Mol Biol* **817**: 69–91.
- Cruet-Hennequart S, Villalan S, Kaczmarczyk A, O'Meara E, Sokol AM, Carty MP. (2009). Characterization of the effects of cisplatin and carboplatin on cell cycle progression and DNA damage response activation in DNA polymerase eta-deficient human cells. *Cell Cycle (Georgetown, Tex)* **8**: 3039–3050.
- de Castria TB, da Silva EM, Gois AF, Riera R. (2013). Cisplatin versus carboplatin in combination with third-generation drugs for advanced non-small cell lung cancer. *Cochrane Database Syst Rev* CD009256.
- Desai B, Gadhia P. (2012). Chromosomal rearrangements in lymphocytes of head and neck squamous cell carcinoma treated with chemotherapeutic agents. *Neoplasma* **59**: 463–468.
- Duan X, He C, Kron SJ, Lin W. (2016). Nanoparticle formulations of cisplatin for cancer therapy. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* **8**: 776–791.
- Duffell SB, Robinson BA. (1997). Clinical pharmacokinetics and dose optimization of carboplatin. *Clin Pharmacokinet* **33**: 161–183.
- Fennell DA, Summers Y, Cadranell J, Benepal T, Christoph DC, Lal R, Das M, Maxwell F, Visseren-Grul C, Ferry D. (2016). Cisplatin in the modern era: The backbone of first-line chemotherapy for non-small cell lung cancer. *Cancer Treat Rev* **44**: 42–50.

- Ghosh P, Roy SS, Chakraborty P, Ghosh S, Bhattacharya S. (2013). Effects of organoselenium compound 2-(5-selenocyanato-pentyl)-benzo[de]isoquinoline 1,3-dione on cisplatin induced nephrotoxicity and genotoxicity: an investigation of the influence of the compound on oxidative stress and antioxidant enzyme system. *Biometals* **26**: 61–73.
- Gonzalez Cid M, Mudry M, Larripa I. (1995). Chromosome damage induced by carboplatin (CBDCA). *Toxicol Lett* **76**: 97–103.
- Hah SS, Stivers KM, de Vere White RW, Henderson PT. (2006). Kinetics of carboplatin-DNA binding in genomic DNA and bladder cancer cells as determined by accelerator mass spectrometry. *Chem Res Toxicol* **19**: 622–626.
- Hongo A, Seki S, Akiyama K, Kudo T. (1994). A comparison of *in vitro* platinum-DNA adduct formation between carboplatin and cisplatin. *Int J Biochem* **26**: 1009–1016.
- Hotta K, Matsuo K, Ueoka H, Kiura K, Tabata M, Tanimoto M. (2004). Role of adjuvant chemotherapy in patients with resected non-small-cell lung cancer: reappraisal with a meta-analysis of randomized controlled trials. *J Clin Oncol* **22**: 3860–3867.
- Chibber R, Ord MJ. (1989). The mutagenic and carcinogenic properties of three second generation antitumour platinum compounds: a comparison with cisplatin. *Eur J Cancer Clin Oncol* **25**: 27–33.
- Khabour OF, Alsatari ES, Azab M, Alzoubi KH, Sadiq MF. (2011). Assessment of genotoxicity of waterpipe and cigarette smoking in lymphocytes using the sister-chromatid exchange assay: a comparative study. *Environ Mol Mutagen* **52**: 224–228.
- Khabour OF, Alzoubi KH, Mfady DS, Alasseiri M, Hasheesh TF. (2014). Tempol protects human lymphocytes from genotoxicity induced by cisplatin. *Int J Clin Exp Med* **7**: 982–988.
- Kralovanszky J, Prajda N, Kerpel-Fronius S, Gal F, Kiss F. (1988). Comparison of intestinal toxic effects of platinum complexes: cisplatin (CDDP), carboplatin (CBDCA), and iproplatin (CHIP). *Cancer Chemother Pharmacol* **21**: 40–44.
- Mhaidat NM, Alzoubi KH, Khabour OF, Alawneh KZ, Raffee LA, Alsatari ES, Hussein EI, Bani-Hani KE. (2016). Assessment of genotoxicity of vincristine, vinblastine and vinorelbine in human cultured lymphocytes: a comparative study. *Balkan J Med Genet* **19**: 13–20.
- Monchamont C, Auberdic P, Melis A, Afqir S, Pacaut C, Chargari C, Merrouche Y, Magne N. (2011). [Cisplatin or carboplatin, that is the question]. *Bull Cancer* **98**: 164–175.
- Mylonaki-Charalambous E, Mourelatos D, Kotsis A. (1998). A comparative study of the cytogenetic and antineoplastic effects induced by carboplatin in combination with niacin in human lymphocytes *in vitro* and in Ehrlich ascites tumor cells *in vivo*. *Chemotherapy* **44**: 121–128.
- Pastor ML, Laffont CM, Gladiéff L, Chatelut E, Concordet D. (2015). Model-based approach to early predict prolonged high grade neutropenia in carboplatin-treated patients and guide G-CSF prophylactic treatment. *Pharm Res* **32**: 654–664.
- Rjiba-Touati K, Ayed-Boussema I, Soualeh N, Achour A, Bacha H, Abid S. (2013). Antioxidant and antigenotoxic role of recombinant human erythropoietin against alkylating agents: cisplatin and mitomycin C in cultured Vero cells. *Exp Biol Med (Maywood)* **238**: 943–950.
- Sasaki MS. (1980). Chromosome aberration formation and sister chromatid exchange in relation to DNA repair in human cells. *Basic Life Sci* **15**: 285–313.
- Shinkai T, Saijo N, Eguchi K, Sasaki Y, Tamura T, Sakurai M, Suga J, Nakano H, Nakagawa K, Hong WS, et al. (1988). Cytogenetic effect of carboplatin on human lymphocytes. *Cancer Chemother Pharmacol* **21**: 203–207.