Cancer Chemother Pharmacol (2005) 55: 301–305
DOI 10.1007/s00280-004-0847-5

SHORT COMMUNICATION

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Oxaliplatin activity in head and neck cancer cell lines

Received: 29 January 2004 / Accepted: 20 April 2004 / Published online: 21 October 2004

Abstract Oxaliplatin (*cis*-[(1*R*,2*R*)-1,2-cyclohexanediamine-*N*,*N*′] [oxalato(2-)-*O*,*O*′] platinum; Eloxatin) is a third-generation platinum compound with a 1,2-diaminocyclohexane (DACH) carrier ligand, which has a wide spectrum of anticancer activity in vitro systems and has displayed preclinical and clinical activity in a wide variety of tumors. To investigate its in vitro activity against head and neck cancer, we exposed two head and neck cancer cell lines to the compound, created a variant resistant to cisplatin to study cross-resistance to the compound and analyzed the potential radiosensitizing effect of the drug. We report here that oxaliplatin was cytotoxic at similar doses to cisplatin in these cells. There was no cross-resistance to cisplatin, as demonstrated by different IC_{50} values in these cell lines and the sensitivity to oxaliplatin of the cisplatin-resistant cell line. There was an effective radiosensitizer effect of the compound in either cell line. Additional in vitro and in vivo experimentation is warranted in order to support the use of oxaliplatin as a radiosensitizer in head and neck cancer patients.

Keywords Oxaliplatin · Cisplatin · Radiation · Resistance · Radiosensitization

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Introduction

Oxaliplatin (*cis*-[(1*R*,2*R*)-1,2-cyclohexanediamine-*N*,*N*′] [oxalato(2-)-*O*,*O*′] platinum; Eloxatin) is a third-generation platinum compound with a 1,2-diaminocyclohexane (DACH) carrier ligand, which has a wide spectrum of anticancer activity in in vitro systems and has displayed preclinical and clinical activity in a wide variety of tumors [1, 2]. Oxaliplatin, like other platinum compounds, acts primarily by causing interstrand and intrastrand crosslinks in DNA, thereby inhibiting DNA synthesis.

This drug has distinct biochemical, pharmacological and cytotoxic properties which are different from those of cisplatin. Although it forms fewer Pt-DNA adducts it is more efficient than cisplatin per equal number of DNA adducts in inhibiting DNA chain and exhibits similar or greater cytotoxicity in several human tumor cell lines [3]. It appears to have distinct mechanisms of resistance, so platinum-insensitive tumor types may be susceptible to oxaliplatin [4].

Oxaliplatin has shown antitumoral activity in a wide variety of tumors such as ovarian, non-small-cell lung, breast cancer and non-Hodgkin's lymphoma. In addition, there are preclinical and clinical reports of additive and synergistic effects between oxaliplatin and several anticancer drugs including cisplatin in various tumor types [5].

The effect of oxaliplatin in head and neck cancer has been poorly explored. There is only one reported phase II clinical trial using oxaliplatin for this neoplasm in combination with Taxol [6]. In this work we analyzed the in vitro effects of oxaliplatin in two head and neck cancer cell lines, focusing in its radiosensitization effect and cross-resistance with cisplatin.

Methods

Cell culture

KB and Hep2 cancer cells lines were obtained from the American Type Culture Collection (ATCC). The cell lines were maintained as a monolayer at 37°C, and cultured in Dulbecco's modified Eagle's medium containing 10% (v/v) fetal bovine serum in a humidified atmosphere of 5% (v/v) carbon dioxide in air.

Resistance to cisplatin

The cell lines were exposed to an initial cisplatin concentration of 0.1 μM. Surviving cells were exposed to successive doublings of cisplatin concentration every 2–3 weeks up to 1 or 2 μM.

Cellular viability and cytotoxic effects

Cells were seeded in 24- or 96-well chamber dishes and exposed to several concentrations of oxaliplatin (1×10^{-4}–1×10^{-10} M) for 24 h. The medium was changed and viable cells, which remained attached to the dish, were fixed in 70% ethanol at −20°C, washed in PBS and stained with crystal violet (1% in water). After washing, the stain was solubilized in 33% acetic acid and the absorbance determined in a microplate reader at 570 nm. The analysis was performed at least in triplicate in four independent experiments.

Clonogenicity assay

Cells were exposed to several concentrations of oxaliplatin (1×10^{-4}–1×10^{-10} M) for 24 h. The cells were then trypsinized and seeded in 100-mm Petri dishes at low density (100 cells/dish) for 14 days in an atmosphere containing 7% CO_2 in an incubator at 37°C. The cells were then fixed, stained with crystal violet and colonies counted using an inverted microscope, as described in previously [7, 8]. Each experiment was performed in triplicate.

Clonogenicity assay and radiation enhancement ratios

Cells were exposed to several concentrations of oxaliplatin (1×10^{-4}–1×10^{-10} M) for 24 h. The medium was aspirated to leave only a 1-mm film and the cells exposed to the shown doses of gamma radiation. The cells were tripsinized and seeded in 100-mm petri dishes at a density of 100 cells/dish for 14 days in an atmosphere containing 7% CO_2 in an incubator at 37°C. The cells were then fixed, stained with crystal violet and colonies counted using an inverted microscope. The enhancement ratio was defined as the mean inactivation dose for the control/mean inactivation dose for the drug-treated cells. An enhancement ratio greater than one indicates that the drug is acting as a radiosensitizer [8, 9].

Results

Exposure of cell lines derived from oral (KB) and laryngeal (Hep2) carcinomas to oxaliplatin resulted in a concentration-dependent viability decrease as shown in Fig. 1. The IC_{50} values (Table 1) showed that KB cells were more susceptible to oxaliplatin than Hep2 cells. The opposite was true for cisplatin, since KB cells showed an IC_{50} of 147.3 μM while Hep2 showed an IC_{50} of 19.2 μM. These results are in accordance with the reported different mechanisms of intrinsic resistance for the two platin compounds in other cell types [10].

In order to confirm these results for an extrinsic resistance model, we created a variant cell line derived from Hep2 cells. We selected these cells since they were the most sensitive to the cisplatin. This line was established by culturing the cells with increasing concentrations of the drug, as described in "Methods." The new cell line Hep2R, was able to grow in the presence of 1 μM cisplatin. The resistant cell line did not acquire cross-resistance to oxaliplatin (Fig. 2).

Finally, we sought to determine whether oxaliplatin could be an effective radiosensitizer. Figure 3 shows that oxaliplatin synergized with radiation to induce reproductive cell death in both cell lines, although the effect was less pronounced in KB cells. The enhancement ratios for Hep2 were 1.31 ± 0.02 and 1.60 ± 0.03 at 2 μM and 4 μM, respectively. For KB cells, the enhancement ratios were 1.21 ± 0.04 and 1.37 ± 0.02 for 2 μM and 4 μM, respectively.

Discussion

Oxaliplatin is a novel platinum analogue with a wide spectrum of anticancer activity [1, 11]. We report here the in vitro activity of oxaliplatin against two human cell lines derived from the mouth and larynx, exploring also the potential of oxaliplatin as a radiosensitizer.

Oxaliplatin was found to be active against KB and Hep2 cell lines with IC_{50} values of 93.1 μM and 184.6 μM, respectively. In accordance with previous reports for cell lines derived from other tumors, the cytotoxic activity of oxaliplatin did not correlate with the activity of cisplatin in the same cell lines. KB cells were more sensitive to oxaliplatin and less sensitive to cisplatin than Hep2 cells. This response agrees with the results obtained by Soulie et al. in the same cell line [12]. It is noteworthy that Mukai et al. found that an isolated clone of KB cells resistant to cisplatin (KCP-4 cells) have cross-resistance to oxaliplatin [13]. This difference is likely due to the procedure employed for the isolation of resistant cells, since several steps were used by them [14],

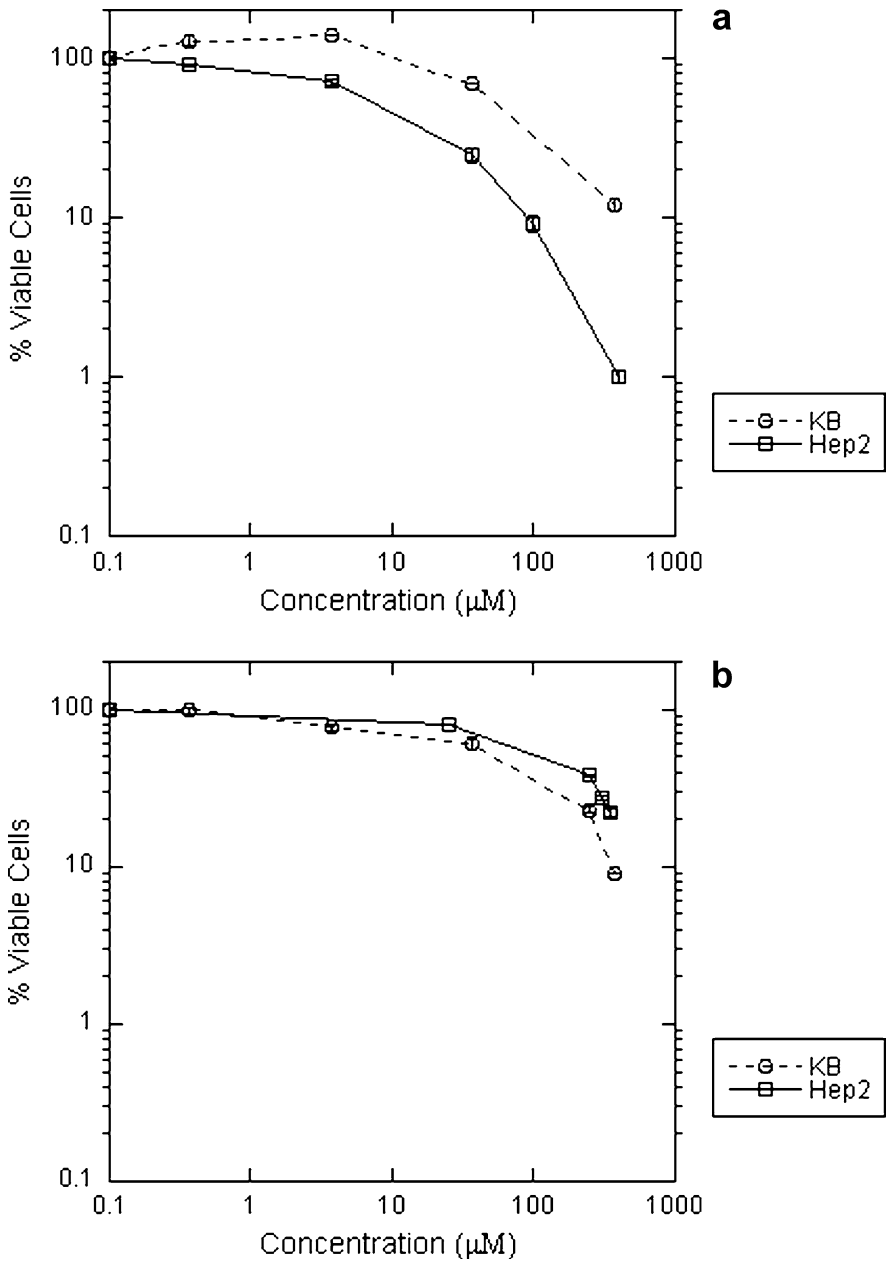

Fig. 1 Percent viability of KB and HepG2 cells exposed to different concentrations of (**a**) cisplatin and (**b**) oxaliplatin for 24 h. The viability was determined by crystal violet staining as described in "Methods." The analyses were performed at least in triplicate in four independent experiments

as opposed to the straightforward approach used by us. Alternatively, it could be due to the cell line employed, as some of the KB cells used routinely in some laboratories are contaminated with cervical cancer cells [15]. Our results indicate that in both cell lines there is little or no cross-resistance between oxaliplatin and intrinsic cisplatin resistance.

Table 1 IC_{50} values of cisplatin and oxaliplatin in KB and Hep2 cell lines following exposure for 24 h

Cell line	IC_{50} (μM)	
	Cisplatin	Oxaliplatin
Hep2	19.2 ± 1.3	184.6 ± 5.4
KB	147.3 ± 5.2	93.1 ± 3.4

Cisplatin and oxaliplatin induce a cell cycle block in the G_2/M phase [16] and generate DNA lesions as interstrand DNA crosslinks (ISC) and DNA-protein crosslinks (DPC) [17]. Although the molecular mechanism(s) providing the difference in resistance remain largely unknown, some in vitro studies underline the importance of DNA repair. The adducts formed by both drugs are repaired with similar kinetics, but oxaliplatin is more efficient than cisplatin per equal number of DNA adducts in inhibiting DNA chain [3]. The differences in the structure of the adducts produced by cisplatin and oxaliplatin are consistent with the observation that they are differentially recognized by the

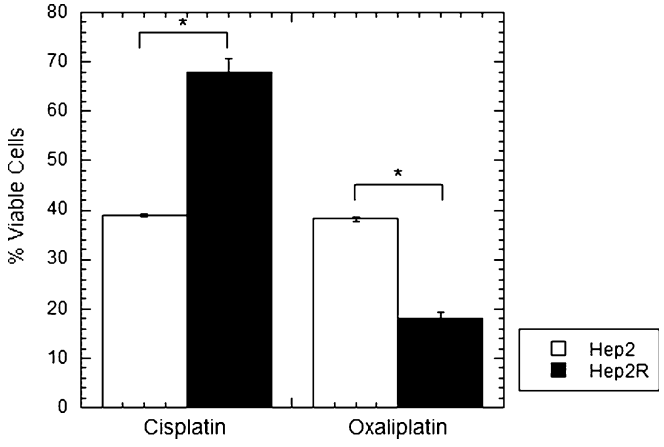

Fig. 2 Percent viability of Hep2 (*empty bars*) and Hep2R cells (*solid bars*) following exposure to cisplatin (20 μ*M*) or to oxaliplatin (20 μ*M*). The viability was determined by crystal violet staining as described in "Methods." The analyses were performed at least in triplicate in four independent experiments. $*P < 0.05$ by Student's *t*-test

DNA mismatch repair system [18]. Studies have shown that the loss of DNA mismatch repair results in resistance to cisplatin but not to oxaliplatin [18]. For example, the DNA mismatch repair system plays an important part in the recognition of cisplatin adducts, and activation of both the JNK and c-Abl kinases in response to cisplatin damage is dependent on the detector function of the DNA mismatch repair proteins. In contrast, this detector does not respond to oxaliplatin adducts [19]. Our results with the cisplatin-resistant cell lines support this notion, since there was also no cross-resistance between oxaliplatin and extrinsic cisplatin resistance. These two variant cell lines could be a tool to investigate the mechanism(s) involved in the intrinsic and extrinsic resistance to cisplatin and oxaliplatin.

Oxaliplatin has been used as a radiosensitizer for rectal [20, 21] and esophageal cancer [22] and its potential for use in other cancer types has been analyzed in vitro using colorectal [23] and cervical cancer cell lines. In the present study we found that oxaliplatin was a good radiosensitizer in these cell lines, with enhancement ratios above 1.2. Combinatorial studies are being undertaken in our laboratory in order to ascertain the utility of oxaliplatin in newer chemotherapy schemes.

Our results provide support for clinical studies using oxaliplatin in some tumors resistant to cisplatin such as head and neck and lung cancers and germ cell tumors. Furthermore, oxaliplatin is currently considered one of the best drugs to treat colorectal cancer [24], so it may be worthwhile to investigate the effectiveness of oxaliplatin in combination with radiotherapy in this tumor in clinical trials.

Acknowledgements We want to thank Ms Gisela Olvera for her kind help. This work was partly supported by grants 42039-A1 (to V. Maldonado) and 42040-A1 (to J. Meléndez-Zajgla) from Consejo Nacional de Ciencia y Tecnología, México.

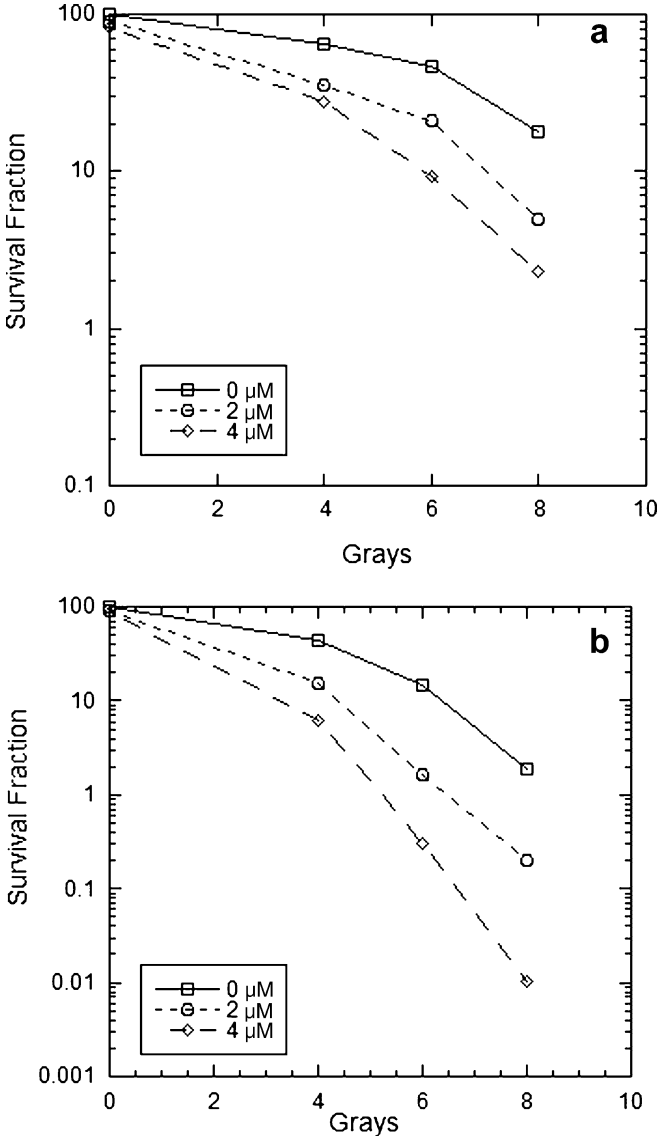

Fig. 3 Radiosensitizing effect of oxaliplatin. KB (**a**) and Hep2 (**b**) cells were exposed to different concentrations of the drug for 24 h and irradiated at the indicated doses, and clonogenicity assays performed to estimate the surviving fraction

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