Cancer Chemother Pharmacol (1997) 40: 94–97

SHORT COMMUNICATION

Atsunori Nitta · Yong-Suk Chung · Bunzo Nakata
Masakazu Yashiro · Naoyoshi Onoda · Kiyoshi Maeda
Tetsuji Sawada · Michio Sowa

Establishment of a cisplatin-resistant gastric carcinoma cell line OCUM-2M/DDP

Received: 16 August 1996 / Accepted: 16 November 1996

Abstract A *cis*-diamminedichloroplatinum (CDDP)-resistant scirrhous gastric cancer cell line, OCUM-2M/DDP, was established by chronic exposure of cells of the parent scirrhous gastric cancer cell line, OCUM-2M, to CDDP at progressively increasing concentrations. The OCUM-2M/DDP cell line had an 11.3-fold higher level of resistance relative to its parent cell line as determined by a succinate dehydrogenase inhibition test. The biological and biochemical characteristics of the resistant and parent cell line were compared. There were differences in the modal chromosome number and DNA index, suggesting that some alterations of the DNA in the CDDP-resistant cells had occurred. Neither the parent nor resistant cell line expressed *mdr*-1 mRNA. After exposure to CDDP for 4 h, the intracellular platinum content of OCUM-2M cells was significantly higher than that of OCUM-2M/DDP cells (51.9 ± 1.8 vs 16.4 plus 1.0 ng/mg protein, mean $\pm$ SD, respectively). The GSH levels in OCUM-2M cells and OCUM-2M/DDP cells were 3.5 ± 1.0 μg/mg protein and 16.8 ± 1.2 μg/mg protein, respectively. These levels were also significantly different. These findings suggest that the possible mechanisms of acquired resistance to CDDP in OCUM-2M/DDP cells may be a decrease in intracellular CDDP accumulation and detoxication by GSH. This OCUM-2M/DDP cell line could be used in further investigations of the mechanism of CDDP resistance in gastric cancer.

Key words Gastric carcinoma · Cisplatin resistance · Cell line · Glutathione

Introduction

Cisplatin (*cis*-diamminedichloroplatinum) was developed by Rosenberg [17] in the 1960s and was initially used in the treatment of head and neck, uterine and bladder cancers [11]. More recently, it has also been used in the treatment of gastric cancer and other gastrointestinal malignancies and has produced high response rates. However, tumors that initially respond to treatment often acquire resistance to cisplatin during long-term use, and this is a major problem in chemotherapy [3, 7]. To investigate the mechanism of cisplatin resistance, we established a cisplatin-resistant scirrhous gastric carcinoma cell line (OCUM-2M/DDP) and compared its biological and biochemical properties with those of the parent cell line (OCUM-2M).

A. Nitta · Y-S. Chung · B. Nakata (✉) · M. Yashiro · N. Onoda · K. Maeda · T. Sawada · M. Sowa
First Department of Surgery, Osaka City University Medical School, 1-5-7 Asahi-machi, Abeno-ku, Osaka 545, Japan
Tel. 6–645–2146; Fax 6–646–6450

Materials and methods

Cells

The OCUM-2M cell line was established in our laboratory from a primary scirrhous gastric carcinoma [13]. OCUM-2M cells are round and grow as free-floating single cells or clusters, with a doubling time of 18.1 h (passage 200).

Induction of cisplatin resistance

The cells were suspended in culture dishes (Falcon No. 3002, Falcon Co., Lincoln Park, NJ, USA) containing Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated fetal bovine serum and were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Cisplatin-resistant cells were established by exposure to increasing concentrations of cisplatin. OCUM-2M cells were initially incubated in DMEM containing 1 μg/ml cisplatin (Nippon Kayaku, Tokyo, Japan), and the cells that proliferated were repeatedly subcultured in DMEM containing increasing concentrations of the drug (a 20% increment each time) [6]. Cells were obtained that grew exponentially in the presence of 5 μg/ml cisplatin, and were designated as OCUM-2M/DDP cells.

Measurement of sensitivity to cisplatin

The sensitivity of OCUM-2M/DDP cells to cisplatin was measured using a succinate dehydrogenase inhibition test [8]. The concentration of cisplatin that produced 50% inhibition of growth was designated as the 50% inhibitory concentration (IC_{50}).

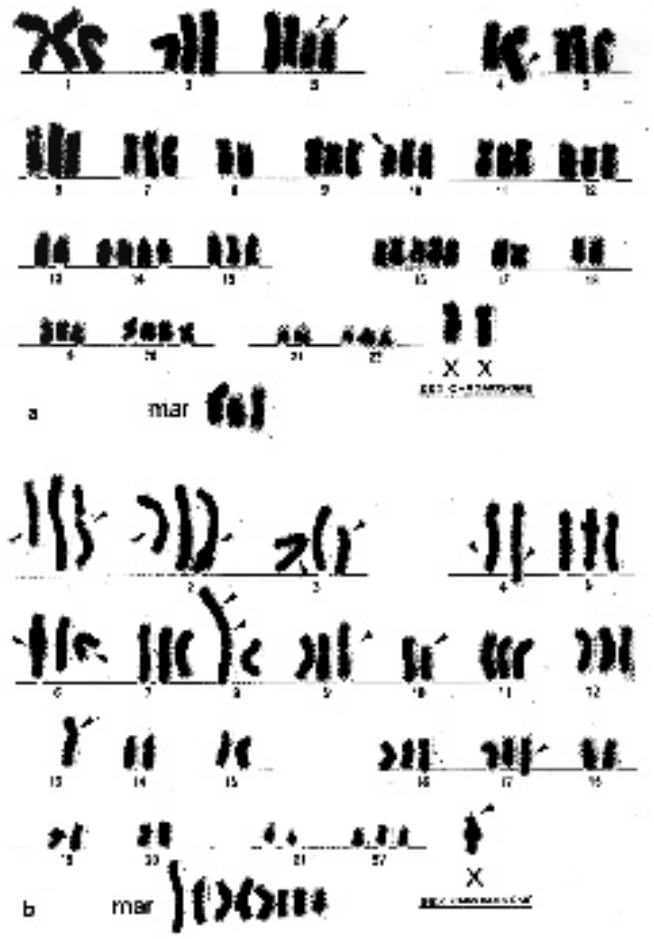

Fig. 1a, b G-banded karyotypes. **a** OCUM-2M, **b** OCUM-2M/DDP

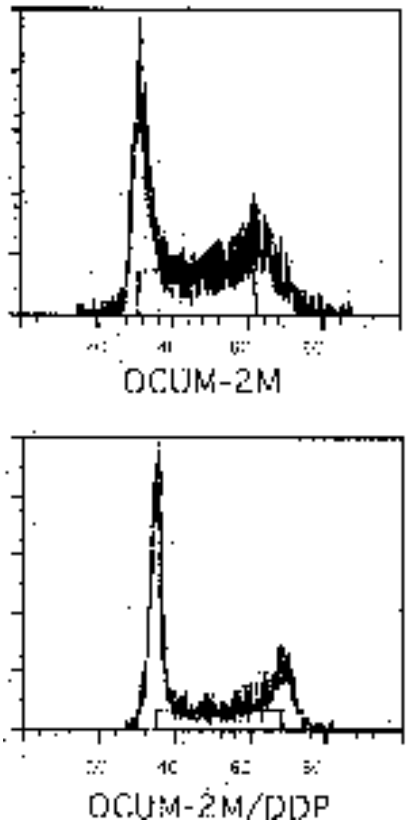

Fig. 2a, b DNA ploidy patterns. **a** OCUM-2M. The percentages of G_1, S and G_2/M are 33.6%, 52.5% and 13.9%, respectively. **b** OCUM-2M/DDP. The percentages of G_1, S and G2/M are 46.3%, 32.85, and 20.9%, respectively

Determination of the doubling time

The doubling time was determined by a replicate culture method [18].

Chromosome analysis

The karyotype was analyzed by the G-banding technique [19]. The modal chromosome number was obtained from 20 metaphases.

Determination of the nuclear DNA content

The nuclear DNA content was measured in 10^4 cells by flow cytometry (FACScan, Becton Dickinson Labware, Mountain View, Calif., USA), and the DNA histograms were used to determine the DNA ploidy pattern and DNA index. Normal human peripheral blood lymphocytes were used as the control, with the lymphocyte G_1 peak being designated as 2C.

Expression of mdr-1 mRNA

Expression of the *mdr*-1 gene, which encodes membrane P-glycoprotein [2], was examined by a reverse transcription-polymerase chain reaction method.

Measurement of intracellular platinum

Cells in the log phase of growth were suspended in medium at a density of 1×10^6/ml and incubated for 4 h in the presence of 10 μg/ml cisplatin at 37 °C in a humidified atmosphere containing 5% CO_2. The intracellular platinum level was determined by atomic absorption spectrophotometry [9, 16].

Measurement of intracellular reduced glutathione

The intracellular reduced glutathione (GSH) level was determined by the method of Tietze [20].

Results

Cisplatin resistance

The IC_{50} value of cisplatin was 0.8 μg/ml for OCUM-2M cells compared with 9.0 μg/ml for OCUM-2M/DDP cells. Thus, OCUM-2M/DDP cells were 11.3-fold more resistant to cisplatin than their parent cells.

Morphology

Under the phase-contrast microscope, both OCUM-2M and OCUM-2M/DDP cells were seen to be round and floating in the culture medium, and were morphologically indistinguishable.

Doubling time

The doubling time of OCUM-2M cells was 18.1 h and that of OCUM-2M/DDP cells was slightly longer at 22.0 h.

Modal chromosomal number and DNA index

The modal number of chromosomes was 70 for OCUM-2M cells and 64 for OCUM-2M/DDP cells (Fig. 1). The DNA ploidy pattern was aneuploid for both cell lines. The DNA index was 1.59 for OCUM-2M cells and 1.78 for OCUM-2M/DDP cells (Fig. 2).

Expression of *mdr-1* mRNA

Expression of mRNA for the mdr-1 gene was not detected in either the parent or resistant cell line.

Intracellular platinum content

After exposure to cisplatin for 4 h, the intracellular platinum content in OCUM-2M cells was 51.9 ± 1.8 ng/mg protein (mean $\pm$ SD, $n = 4$) and was significantly lower in OCUM-2M/DDP cells at 16.4 ± 1.0 ng/mg protein ($n = 4$).

Intracellular GSH content

The GSH level was 3.5 ± 1.0 μg/mg protein ($n = 4$) in OCUM-2M cells and significantly higher at 16.8 ± 1.2 μg/mg protein ($n = 4$) in OCUM-2M/DDP cells.

Discussion

Cisplatin has recently been shown to have an excellent antitumor effect against gastric cancer and is now increasingly used in the treatment of advanced and recurrent gastric cancer [15], but the acquisition of resistance is also a significant problem. However, there are few reports of the establishment of cisplatin-resistant gastric cancer cell lines.

The cisplatin-resistant cell line that we established from a scirrhous gastric cancer cell line showed resistance 11.3-fold higher to the drug than the parent cells as well as good growth kinetics with a similar doubling time to its parent. The parent and resistant cell lines showed differences in their modal chromosome number mode and DNA index. These differences are interesting since they suggest that cellular alterations had occurred at the DNA level during selection of the resistant cells. Although the actual genetic changes involved in the induction of cisplatin resistance require clarification, the resistance of our OCUM-2M/DDP cells remained stable after 6 months of incubation in cisplatin-free medium, suggesting that irreversible changes had occurred at the DNA level. OCUM-2M/DDP cells isolated from OCUM-2M human gastric cancer cells therefore appear to be a cisplatin-resistant gastric cancer cell line.

After exposure to cisplatin, the intracellular content of the drug was significantly lower in the resistant cells than in the parent cells, suggesting decreased accumulation (decreased influx or increased efflux) of cisplatin in the resistant cells. It has been reported that the ATP-dependent efflux mechanism mediated by membrane-associated P-glycoprotein [5] does not appear to be involved in reducing the intracellular cisplatin content [14]. In addition, expression of the *mdr*-1 gene encoding P-glycoprotein was not detected in either OCUM-2M or OCUM-2M/DDP cells, so the involvement of this mechanism seems unlikely. GSH is a tripeptide composed of glycine, cysteine and glutamate, and is thought to play a central role in the cellular detoxification of alkylating agents and cisplatin. The GSH level was significantly higher in OCUM-2M/DDP cells than in OCUM-2M cells. There are several other reports of elevated GSH levels in cultured cisplatin-resistant cells [1, 4, 12]. In clinical tumor specimens, the intracellular GSH level has also been found to be elevated after drug resistance develops [10]. These findings suggest that GSH is deeply involved in the acquisition of cisplatin resistance.

In conclusion, both decreased accumulation of cisplatin and increased detoxification via GSH may be involved in the acquisition of cisplatin resistance by OCUM-2M/DDP cells. In addition, the OCUM-2M/DDP cell line may be a useful in vitro model for studies on the resistance mechanism and for overcoming the cisplatin resistance of gastric cancer.

References

1. Bedford P, Shellard SA, Walker MC, et al (1987) Differential expression of collateral sensitivity or resistance to cisplatin in human bladder carcinoma cell line pre-exposed in vitro to either x-irradiation or cisplatin. Int J Cancer 40:681–686
2. Chen C-J, Clark D, Ueda K, et al (1990) Genomic organization of the human multidrug resistance (MDR1) gene and origin of P-glycoproteins. J Biol Chem 265:506
3. Hospers GAP, Mever L (1990) A study of human small cell lung carcinoma (hSCLC) cell lines with different sensitivities to detect relevant mechanisms of cisplatin (CDDP) resistance. Int J Cancer 46:138–144
4. Hosper SGAP, Mulder NH, de Jong B, et al (1988) Characterization of a human small cell lung carcinoma cell line with acquired resistance to cis-diamminedichloroplatinum (II) in vitro. Cancer Res 48:6803–6807

5. Juliano RL, Ling V (1976) A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 455:152–162
6. Kikuchi Y, Iwano I, Miyauchi M, et al (1988) The mechanism of acquired resistance to cisplatin by a human overian cancer cell line. Jpn J Cancer Res 79:632–635
7. Kikuchi Y, Iwano I, Miyauchi M, et al (1990) Possible mechanisms of resistance to cis-diamminedichloroplatinum (II) of human overian cancer cells. Cancer Res 81:701–706
8. Kondo T, Imamura T, Ichihashi H (1996) In vitro test for sensitivity of tumor to carcinostatic agents. Gann 57:113–121
9. LeRoy AF, Wehling ML, Sponseller HL, et al (1972) Analysis of platinum in biological materials by flameless atomic absorption spectrophotometry. Biochem Med 18:184–191
10. Lewis AD, Hayes JD, Wolf CR (1988) Glutathione and glutathione-dependent enzymes in ovarian adenocarcinoma cell lines derived from a patient before and after the onset of drug resistance: intrinsic differences and cell cycle effects. Carcinogenesis 9:1283–1287
11. Loehrer PJ, Einhorn LH (1984) Cisplatin. Ann Intern Med100: 704–713
12. Mansouri A, Henle KJ, Benson AM, et al (1989) Characterization of a cisplatin-resistant subline of murine RIF-1 cells and reversal of drug resistance by hyperthermia. Cancer Res 49:2674–2678
13. Masakazu Yashiro, Yong-Suk Chung, Michio Sowa (1994) Role of orthotopic fibroblasts in the development of scirrhous gastric carcinoma. Jpn J Cancer Res 85:883–886
14. Nooter K, Herweijer H (1991) Multidrug resistance (mdr) genes in human cancer. Br J Cancer 63:663–669
15. Ohtsu A, Yoshida S, Saito D, et al (1991) An early phase II study of 5-fluorouracil combined with cisplatin as a second line chemotherapy against metastatic gastric cancer. Jpn J Clin Oncol 21:120–124
16. Remtulla MA, Soldin SJ (1982) Analysis of cisplatin in plasma and the ultrafiltrate of whole blood. Clin Chem 28:1572
17. Rosenberg B (1985) Fundamental studies with cisplatin. Cancer 55:2303–2316
18. Sanford KK, Earle WR, Evans VJ, et al (1951) The measurement of proliferation in tissue cultures by enumeration of cell nuclei. J Natl Cancer Inst II:773–775
19. Seabright M (1971) A rapid banding technique for human chromosomes. Lancet 2:971–972
20. Tietze F (1969) Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione. Anal Biochem 27:502–522