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In vitro colony inhibition of carboplatin against stomach and lung cancer cell lines in comparison with cisplatin*

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Summary. The effects of carboplatin and cisplatin on colony formation in stomach and lung cancer cell lines were examined and compared. The colony-inhibitory activity of carboplatin against stomach and lung cancer cell lines was similar to that of cisplatin when one-tenth of the peak plasma concentration of each drug was used ($r=0.80$). One of the four stomach cancer cell lines was sensitive to carboplatin although all the stomach cancer cell lines were resistant to cisplatin. Of the three small cell lung cancer cell lines tested, two were sensitive to both carboplatin and cisplatin, and only one cell line (N857) was resistant to cisplatin; all the non-small cell cancer cell lines tested were resistant to both drugs. On the basis of these preliminary results, we suggest that carboplatin has potential therapeutic activity against stomach cancer and should be evaluated carefully from this aspect.

Introduction

In recent years, a high response rate to cisplatin (CDDP), either alone or, more often, in combination with other agents has been achieved in a variety of cancers [6]. Unfortunately, serious side effects of CDDP, such as renal toxicity, nausea and vomiting, have limited its clinical usefulness, and a great deal of effort has been devoted to the development of compounds with either equivalent or higher antitumor activity and lower toxicity than CDDP. Carboplatin (CBDCA), an analogue of CDDP, has proven to be one of the most promising agents among them [2]. Phase I studies on CBDCA have demonstrated that CBDCA has little or no nephrotoxicity and less gastrointestinal toxicity than the parent compound CDDP, although CBDCA has the one disadvantage of myelosuppression [3].

CBDCA was reported to have no therapeutic effect against stomach cancer in a phase II trial, the only one reported [7] as far as we know. On the other hand, pharmacological studies on CBDCA have demonstrated that the efficacy and spectrum of activity of CBDCA are similar to those of CDDP [1, 2], which has some activity against stomach cancer [8, 15]. This study was conducted to evaluate the potential value of CBDCA against stomach cancer using four established stomach cancer cell lines and a colony assay. Additional experiments were conducted with three small cell lung cancer (SCLC) and five non-small cell lung cancer (NSCLC) cell lines, because of the previously reported sensitivity of SCLC and resistance of NSCLC to CBDCA. We also examined the effect of CDDP against stomach and lung cancer cell lines to compare the effect of CBDCA with that of CDDP.

Material and methods

Tumor cell lines. Twelve human tumor cell lines (four stomach cancer, five NSCLC and three SCLC) were used in the colony assay. Stomach cancer cell lines (KATO III, MKN74, MKN28, and MKN45) were supplied by the Japanese Antibody Co. NSCLC cell lines, kindly provided by Prof. Y. Hayata, Tokyo Medical College, were PC-1 and PC-3 (squamous cell carcinomas), PC-7 and PC-9 (adenocarcinomas), and PC-13 (large cell carcinoma). SCLC cell lines (N231, N857 and H69) were established at the National Cancer Institute, USA and obtained from Prof. Y. Shimosato, National Cancer Center Research Institute. The cells were propagated in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (Gibco), penicillin 100 units/ml and streptomycin 100 μg/ml (RPMI-FCS) at 5% CO_2, in a highly humidified incubator at 37°C. The doubling times in four stomach cancer, five NSCLC and one SCLC (H69) cell lines ranged from 20 h to 36 h. The doubling times for the two SCLC (N231 and N857) were approximately 72 h [12].

Colony assay. The culture system used was modified from that devised by Hamburger and Salmon [4]. Briefly, tumor cells were harvested from the culture flasks, washed with RPMI medium, and counted with a Coulter counter. Viability of the tumor cells was evaluated by the trypan blue dye exclusion test (>95% of cell viability). One milliliter of tumor cell suspension (1×10^5 cells/ml for PC series, H69, and stomach cancer cell lines, and 3×10^5 cells/ml for N231 and N857) in RPMI-FCS with 0.3% agar (Bacto, Difco, Detroit, Mich) was pipetted onto 1 ml underlayer in

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a 35-mm Falcon 1008 plastic petri dish (Falcon plastic, Oxford, Calif). The underlayer contained 0.5% agar on enriched McCoy's 5A medium (Gibco). Enriched McCoy's 5A medium consisted of 40 ml 10% heat-inactivated fetal calf serum, 20 ml 5% heat-inactivated horse serum (Gibco), 4 ml 2.2% Na pyruvate, 4 ml 200 m*M* glutamine, and 0.8 ml 2.1% serine (Wako Pure Chemical Industry, Osaka, Japan) mixed with 400 ml McCoy's 5A medium. After plating, the tumor cells were inspected under the inverted microscope to confirm that there was no tumor cell clump in the petri dish and then incubated at 37° C in 5% CO_2 in a highly humidified incubator for 10–21 days.

In vitro exposure of tumor cells to drugs. The chemotherapeutic agents used were CBDCA (Bristol Myers Co., New York, USA) and CDDP (500 μg/ml, Nihon Kayaku, Tokyo, Japan). Immediately before use, the dissolved CBDCA and the original CDDP were diluted to the appropriate concentrations with RPMI-FCS. Tumor cells were mixed with 3 ml different final concentrations (0.1–100.0 μg/ml: for stomach cancer cell lines, 0.2, 1.0, 2.0, 5.0 and 10.0 μg/ml; for lung cancer cell lines, 0.1, 0.3, 1.0, 3.0, 10.0 and 100.0 μg/ml) of CBDCA or CDDP solution containing 0.3% agar, and 1 ml of the tumor cell suspension was plated onto the underlayer as described above. Each test was performed in triplicate. After 10–21 days of incubation the colonies were counted by an automatic particle counter (CP-2000, Shiraimatsu Instrument, Osaka, Japan). Colonies larger than 50 μm in diameter were regarded as positive. The percentage survival of colony was calculated from the following formula:

$$\frac{\text{Mean of colony counts in three test dishes}}{\text{Mean of colony counts in three control dishes}} \times 100$$

Each experiment was repeated three times.

Comparison of drug sensitivity. IC_{50} values were determined graphically after obtaining a dose-response curve for each tumor cell line. The in vitro cytotoxicity of CBDCA and CDDP was evaluated by the ratio of one-tenth of the peak plasma concentrations (1/10 ppc) to the IC_{50}. Based on the previous pharmacological studies, CBDCA 2.0 μg/ml and CDDP 0.2 μg/ml were used in this study as 1/10 ppc [5, 9, 11]. Colony inhibitions by CBDCA and CDDP were compared at 1/10 ppc. The relationship of sensitivity between CBDCA and CDDP was also evaluated after classifying sensitive (< 50% of colony survival at 1/10 ppc) and resistant cell groups.

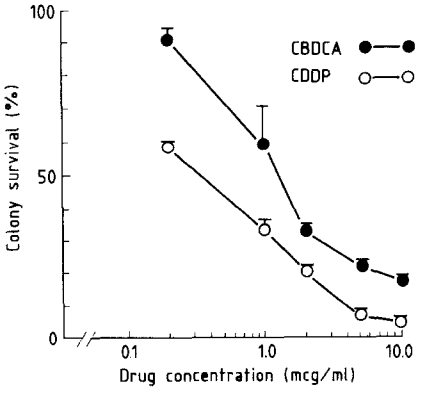

Fig. 1. Dose-response curves of a stomach cancer cell line (MKN-45) to CBDCA and CDDP. Each *point* represents the mean of three determinations ± SD

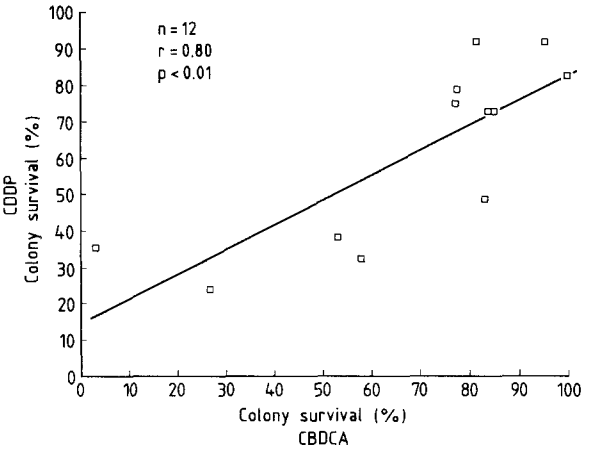

Fig. 2. Comparison of colony survival for CBDCA and CDDP

Results

In vitro cytotoxicity of CBDCA and CDDP against stomach cancer cell lines

The IC_{50}s of CBDCA were significantly higher than those of CDDP in all stomach cancer cell lines. However, in vitro cytotoxicity of CBDCA was similar to that of CDDP when compared at 1/10 ppc to IC_{50} for each cell line. One of the four stomach cancer cell lines (MKN-45) proved to be sensitive to CBDCA (31% of colony survival at 1/10 ppc) (Fig. 1), while all the stomach cancer cell lines were resistant to CDDP (Table 1).

In vitro cytotoxicity of CBDCA and CDDP against human lung cancer cell lines

Although the IC_{50}s of CBDCA were significantly higher than those of CDDP in all NSCLC and SCLC cell lines, in vitro cytotoxicity of CBDCA and CDDP showed no significant difference when compared at 1/10 ppc to IC_{50} for each cell line ($P > 0.05$). All the SCLC cell lines tested proved to be sensitive to CBDCA. However, one of them (N857) was resistant to CDDP. All five NSCLC cell lines tested were resistant to both CBDCA and CDDP (Table 1).

Correlation of colony-inhibitory effect of CBDCA with that of CDDP

Of the twelve tested, two cell lines sensitive to CDDP were sensitive to CBDCA. Eight of ten cell lines resistant to CDDP were also resistant to CBDCA (Table 1). The sensitivity of each cell line to CBDCA was well correlated with that of CDDP (concordance rate: 0.83). Colony survivals of all cell lines were compared at 1/10 ppc of CBDCA and CDDP (Fig. 2). The in vitro colony-inhibitory effect of CBDCA was correlated closely with that of CDDP ($r = 0.80$).

Table 1. Sensitivity of stomach and lung cancer cell lines to CBDCA and CDDP

Cell lines	CDDP		CBDCA	
	IC_{50} (μg/ml)	Inv. cyt.[a]	IC_{50} (μg/ml)	Inv. cyt.
Stomach Ca.				
KATOIII	0.42	0.48	3.65	0.55
MKN-74	1.52	0.13	10.55	0.19
MKN-28	1.22	0.16	7.80	0.26
MKN-45	0.32	0.63	1.23	1.63
	0.87 ± 0.59[b]	0.35 ± 0.24	5.81 ± 4.17	0.66 ± 0.67
NSCLC				
PC-1	0.85	0.24	4.33	0.47
PC-3	0.50	0.40	4.41	0.45
PC-7	1.80	0.11	4.82	0.42
PC-9	0.95	0.21	4.80	0.42
PC-13	0.90	0.22	5.42	0.37
	1.00 ± 0.48	0.24 ± 0.10	4.76 ± 0.43	0.43 ± 0.04
SCLC				
N231	0.09	2.22	0.34	5.88
N857	0.25	0.80	1.45	1.38
H69	0.07	2.86	0.90	2.22
	0.14 ± 0.10	1.99 ± 1.00	0.90 ± 0.56	3.16 ± 2.39

[a] Inv. cyt. (in vitro cytotoxicity): one-tenth of peak plasma concentration/IC_{50} (> 1: sensitive, < 1: resistant)
[b] Means ± SD

Discussion

CBDCA, the most promising platinum compound of the second generation, is known to have significant therapeutic activity against ovarian, testicular, and head and neck cancer, as well as SCLC, and to be similar to CDDP in the spectrum and efficacy of its antitumor activity. In addition, the higher water solubility and dose of administration of CBDCA, due to less pronounced side effects, such as nausea, vomiting and nephrotoxicity, than seen with CDDP, make an increase in the therapeutic index possible. Although CBDCA has the one disadvantage of frequent myelosuppression, known to be a dose-limiting toxicity, it is usually tolerable [1–3].

Stomach cancer is one of the common cancers for which new effective drugs are urgently needed. However, little has been published about the effect of CBDCA on stomach cancer; we are aware of only one report which states that CBDCA did not induce any responses among 20 patients with advanced stomach cancer [7]. On the other hand, CDDP has been used against stomach cancer, either alone or, more often, in combination chemotherapy, with some increase in response rate [8, 15]. Recently, we have observed one complete remission of stomach cancer treated with CBDCA alone [10], which encouraged us to re-evaluate CBDCA against stomach cancer.

To evaluate the effect of a chemotherapeutic agent on a particular tumor it is reasonable to use a preclinical method, such as HTCA, before conducting clinical trials, especially when the preliminary data have shown that the tumor is rather insensitive to the drug. HTCA is the accepted technique for disease-oriented information about in vitro drug activity, yielding results that correlate closely with the clinical response. When in vitro antitumor activities of two drugs are compared using the HTCA the use of appropriate concentrations is very important, as each drug has a different dose of administration with a different plasma concentration. Previous studies have demonstrated that the use of 1/10 ppc is an appropriate concentration giving results corresponding to clinical response in HTCA with 1-h exposure [13, 14]. Pharmacological studies have demonstrated that average peak plasma concentrations of CBDCA and CDDP were 21.9 ± 3.9 μg/ml and 2.49 ± 0.41 μg/ml when CBDCA 400–450 mg/m^2 and CDDP 80–100 mg/m^2 were administered to the patients as single doses by i. v. injection over 15–60 min [5, 9, 11]. The 1/10 ppc of CBDCA and CDDP were chosen on the basis of these pharmacokinetic studies. These experiments were performed with continuous exposure, which has the advantage of being less traumatic to tumor cells and simpler than a 1-h exposure.

In these experiments, CBDCA showed similar in vitro activity to CDDP against colony formations of stomach and lung cancer cells ($r = 0.80$). It is interesting that one stomach cancer and one SCLC cell line were sensitive to CBDCA (< 50% colony survival) although they were resistant to CDDP. We also confirmed that SCLC cell lines were sensitive to both CBDCA and CDDP and that NSCLC cell lines were resistant to both drugs. This preliminary result suggests that CBDCA has potential therapeutic activity against stomach cancer and should be evaluated carefully for clinical efficacy.

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