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Selective intra-arterial infusion of high-dose cisplatin in patients with advanced head and neck cancer results in high tumor platinum concentrations and cisplatin-DNA adduct formation

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Abstract A group of 23 patients with advanced head and neck cancer were treated with highly selective intra-arterial (IA) cisplatin 150 mg/m^2 delivered rapidly through microcatheters. The systemic effects of cisplatin were neutralized by concurrent administration of sodium thiosulfate. Two-to-threefold higher tumor platinum contents were detected in tumor biopsies after selective IA cisplatin administration compared to historicol controls (treated with 100 mg/m^2 IA). Cisplatin-induced DNA modification in human tumor biopsies was quantitated using the antiserum NKI-A59. High levels of cisplatin DNA adducts were detected which correlated linearly with the tumor platinum content ($r^2 = 0.62$). The addition of radiotherapy to this high dose intensity cisplatin treatment resulted in a 92% complete response (CR) rate (12 of 13 patients achieved a CR). Since no difference in tumor platinum content was detected between patients receiving or not receiving radiotherapy (13 and 10 patients, respectively), but the response rate was substantially different (12 CR and 1 partial response with radiotherapy versus 6 partial and 4 non-responders without radiotherapy), these data suggest that the high platinum levels achieved by selective IA infusion were sufficient to produce enough interaction with radiotherapy to cause a 92% CR rate. Whether this interaction is additive or synergistic is as yet unclear.

Key words Cisplatin · DNA adducts · Intra-arterial infusion

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Introduction

Current treatment of head and neck carcinoma utilizes three major modalities: chemotherapy, radiation therapy (XRT), and surgery. A combined modality program using surgery and XRT is considered "standard" therapy [25]. Conventionally fractionated XRT of advanced head and neck carcinoma yields a 5-year survival probability of $< 30\%$ [6]. The hope for further improvement in the treatment of this disease lies primarily with the use of chemotherapy either before or after XRT and/or surgery. Among the chemotherapeutic agents with activity against head and neck cancer, cisplatin appears to be one of the most effective [10].

The development of resistance to cisplatin is, however, a common problem, and constitutes a major obstacle to the cure of even those head and neck carcinomas that are initially sensitive to this drug. Resistance to cisplatin develops rapidly both in vitro and in vivo, and clinically significant degrees of resistance are present within as few as three exposures to the drug [1, 2]. There is abundant laboratory and clinical data indicating that acquired resistance to cisplatin in solid tumors is of a moderate degree and can be overcome by increasing the dose [23, 26]. Retrospective analyses of human trials using cisplatin-based chemotherapy which correlated dose intensity with response have provided substantial evidence that higher dose intensities result in significantly higher response rates [9]. Therefore, it was our hypothesis that increased drug delivery to the tumor would result in higher tumor

drug concentrations and, consequently, enhanced tumor responses [11].

Recently, a number of studies have demonstrated correlations between tumor response and initial DNA platination in non-tumor cells such as buccal mucosal or white blood cells, suggesting a relationship between the extent of DNA platination that occurs in tumor cells and that in potential surrogate marker cells elsewhere in the body [5, 20]. In spite of the correlations observed, the use of surrogate marker cells for the measurement of platinum content is of limited value in assessing actual tumor drug delivery. In the present study we determined the extent of DNA platination in the tumor itself in biopsies obtained from human head and neck tumors before and 24 h after cisplatin treatment. The biopsies were obtained as part of a phase I/II study directed at developing a novel pharmacological strategy that permits selective and rapid intra-arterial (IA) delivery of extremely high doses of cisplatin to head and neck carcinomas [21]. As part of our efforts to analyze the efficacy of this treatment, we now report on the relationship between tumor platinum content and the extent of DNA platination.

Materials and methods

Treatment of patients with cisplatin

A group of 23 patients with advanced (stage III or IV) head and neck cancer were treated on a phase I/II experimental protocol [21]. Patient characteristics are shown in Table 1. All patients received concurrent IA cisplatin and IV sodium thiosulfate. Cisplatin was dissolved in 400 ml 0.9% saline and infused over 3–5 min (1–2 mg cisplatin/s) through a microcatheter placed angiographically such that it selectively encompassed only the dominant blood supply of the targeted tumor [15, 21, 22]. The cisplatin was administered IA at the time of the arteriogram, ensuring optimal supply to the tumor. Simultaneously with the cisplatin infusion, sodium thiosulfate was infused IV at a dose of 9 g/m^2 dissolved in 300 ml distilled water over 3 min, followed by 12 g/m^2 dissolved in 1 l distilled water over 6 h by continuous infusion using a pump [21, 22]. Seven patients received only cisplatin (150 or 200 mg/m^2 weekly for 4 weeks), 3 patients received tamoxifen 60 mg by mouth daily in addition to cisplatin, and 13 patients received XRT (180–200 cGy/fraction, 5 fractions/week, total dose of 6500–7400 cGy) in addition to the cisplatin treatment. The maximum tolerated cisplatin dose was set at 150 mg/m^2 weekly, as determined in the phase I study (protocol acronym HIAPLAT) [21]. A subsequent trial, known as THIAPLAT, piloted the use of concurrent tamoxifen with the HIAPLAT scheme. The addition of tamoxifen to the HIAPLAT regimen was based on the synergistic interaction between cisplatin and tamoxifen in head and neck carcinoma cell lines [18] and recent clinical studies showing better response rates for the combination [16, 17, 18].

Tumor platinum content

Biopsies were obtained before and 24 h after treatment in all patients. Tumor platinum content was analyzed by atomic absorption spectroscopy [13].

Immunocytochemical analysis of cisplatin-DNA modification

Frozen tissue sections of biopsies of 15 patients were mounted on glass slides (2.6 × 6 cm) coated with ovalbumin (100 µl 0.5%

Table 1 Patient characteristics (*HIAPLAT* 150 or 200 mg/m^2 cisplatin weekly for 4 weeks, *THIAPLAT* 150 mg/m^2 cisplatin plus 60 mg tamoxifen by mouth daily, *RADPLAT* 150 mg/m^2 cisplatin plus XRT (180–200 cGy/fraction, 5 fractions/week, total dosage 6500–7400 cy), *NR* no response, *PR* partial response, *CR* complete response

Patient	Primary site	Stage	Neck nodes	Treatment	Cisplatin dose (mg/m^2)	Response	Tumor platinum conc. (µg/g)	Cisplatin-DNA adducts (a.u.)
1	Hard palate	IV	No	HIAPLAT[a]	200	NR	3.1	13.5
2	Floor of mouth	IV	Yes	THIAPLAT[b]	150	NR	50.7	33.1
3	Floor of mouth	III	Yes	THIAPLAT	150	NR	3.9	19.9
4	Pharynx	IV	Yes	HIAPLAT	150	NR	2.6	
5	Tongue	IV	No	THIAPLAT	150	PR	1.4	1.4
6	R. tonsillar fossa	IV	No	HIAPLAT	200	PR	15.0	
7	Parotid gland	III	yes	HIAPLAT	150	PR	1.7	
8	Base of tongue			HIAPLAT	200	PR	3.1	19.4
9	Tongue	IV	No	HIAPLAT	200	PR	3.8	14.7
10	R. ear canal		No	HIAPLAT	150	PR	3.3	19.4
11	R. tonsillar fossa	IV	Yes	RADPLAT[c]	150	CR	1.9	19.3
12	Base of tongue	IV	Yes	RADPLAT	150	CR	3.6	18
13	Tongue	III	No	RADPLAT	150	CR	19.6	
14	Floor of mouth	IV	Yes	RADPLAT	150	CR	2.0	11.9
15	Base of tongue	IV	Yes	RADPLAT	150	CR	3.8	23.4
16	Tongue			RADPLAT	150	CR	7.4	20.2
17	Floor of mouth	IV	Yes	RADPLAT	150	PR	4.7	17.1
18	Pharynx	IV	Yes	RADPLAT	150	CR	0.6	
19	Floor of mouth	IV	Yes	RADPLAT	150	CR	3.2	
20	Base of tongue	IV	Yes	RADPLAT	150	CR	4	15.1
21	Pharynx	IV	Yes	RADPLAT	150	CR	2.6	18.4
22	Base of mouth	IV	Yes	RADPLAT	150	CR	1.9	
23	Base of tongue	III	No	RADPLAT	150	CR	2.3	

ovalbumin per slide). The immunoperoxidase staining procedure, the immunocytochemical analysis and quantitation were carried out as described previously [5].

Statistics

The correlation coefficient between intratumoral platinum content and nuclear stain was calculated from linear regression analysis, and the Mann-Whitney two-sample test was used to study differences; $P < 0.05$ was considered a significant difference.

Results

Figure 1 shows the mean platinum content (± SEM) in biopsies of 23 patients with squamous cell carcinomas treated with high-dose cisplatin (200 or 150 mg/m^2) by the IA route. Figure 1 includes for comparison the mean platinum content measured by Gouyette et al. [8] in head and neck cancers of patients who had received cisplatin 50 mg/m^2 on days 1 and 2 (total dose 100 mg/m^2) by either the IV or the IA route. Significantly higher platinum contents were attained in the tumors treated selectively with the high-dose cisplatin regimen ($P = 0.001$ and 0.015 for the differences from the conventional dose IV- and IA-treated tumors, respectively). The interpatient variation in platinum content was pronounced in patients treated with the high-dose regimen (Table 1). The platinum content varied between 0.6 and 50.7 µg Pt/g tumor; however, most tumors had platinum contents between 3.1 and 4.9 µg Pt/g tumor.

In addition to the tumor platinum concentrations, we determined the level of drug-induced DNA modification in 15 of the 23 patients. Table 1 shows the platinum content and extent of DNA adduct formation as reflected by nuclear staining as a function of the type of response attained by the patient. Relatively high levels of cisplatin-DNA adducts were detected in the head and neck tumor samples compared to those detected in human buccal cells previously reported by Blommaert et al. [5] from patients who had received conventional doses of cisplatin. Analysis of the relationship between the tumor platinum content and the cisplatin-induced DNA modification demonstrated a linear correlation with a coefficient of 0.62 ($P = 0.019$; Fig. 2).

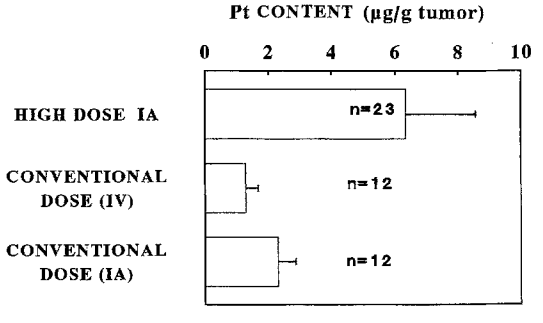

Fig. 1 Mean platinum content in head and neck carcinomas as a function of dose and route of administration (*high dose* 150 mg/m^2 cisplatin, *conventional dose* 100 mg/m^2). The conventional dose data are from Gouyette et al. [8]. Platinum contents differ significantly (Mann-Whitney two-sample test) between high dose and conventional dose IA treatment ($P = 0.015$), and between high dose and conventional dose IV treatment ($P = 0.001$). *Bars* ± SEM

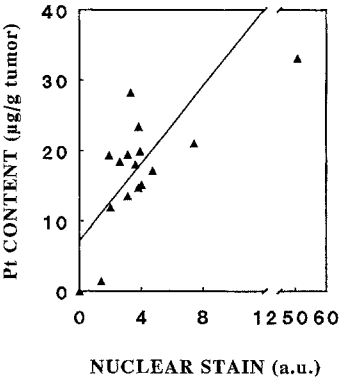

Fig. 2 Correlation between platinum content and extent of DNA adduct formation as measured by nuclear staining intensity in 15 patients who received selective IA cisplatin at a dose of 150 mg/m^2. The correlation coefficient is 0.62 ($P = 0.019$)

It is important to note that not all patients received the same therapy. Of 11 patients achieving either no response or a partial response, 10 received cisplatin alone, while all patients attaining a complete remission received cisplatin plus XRT (12 of 13; one patient treated with cisplatin plus XRT achieved a partial response). However, it is apparent from Table 1 that the distribution of platinum content and extent of DNA adduct formation did not differ between the categories of response. There was no indication that those patients attaining a complete response had a higher level of either platinum content or adduct formation.

Discussion

It has previously been demonstrated that there is a good correlation between the total dose, the resulting platinum content and the extent of DNA adduct formation detectable by immunohistochemical staining in tumor cells (in vitro) and in non-tumor cells in patients [5, 19, 20, 24]. The study reported here indicates that this relationship also holds true in human head and neck tumors as measured in biopsies obtained from patients treated with very high cisplatin dose intensities. The dose intensity of 150–200 mg/m^2 produced 2.5–3-fold higher platinum contents than conventional doses of cisplatin administered by either the IV or the IA route, while the DNA adduct formation in these tumors was 1.5–3-fold higher than measured in non-tumor cells (buccal cells) after conventional doses of cisplatin [5].

It has become clear that multiple mechanisms of cellular resistance to platinum compounds exist [12]. One of the mechanisms involved is intracellular detoxification prior to its binding to DNA, possibly through binding to glutathione or metallothioneins. Such detoxification might be evident as a change in the ratio of the extent of DNA adduct formation to the platinum content, although this has not yet been established experimentally. Most of the samples analyzed in this study demonstrated a high ratio of cisplatin DNA adducts (a.u) to tumor platinum content (μg Pt/g tumor), with values that ranged between 5.5 and 9. This compares with a similar range found in animal studies, where rats bearing peritoneal tumors were treated IP with the maximal tolerated cisplatin dose [14]. In one non-responding patient, however, the ratio was only 0.8, indicating that a relatively small fraction of the cisplatin that entered the cell actually formed DNA adducts and suggesting a high degree of intracellular detoxification in this tumor. In future studies it will be important to determine whether the ratio of adduct formation to platinum content provides any greater power for prediction of response than either platinum content or adduct formation alone.

This study did not permit analysis of the relationship between platinum content or DNA adduct level attained and the probability of response because of the small patient numbers and the fact that the treatment differed between patients. However, within the limitations of the data, there was no apparent difference in platinum content or DNA adduct formation as a function of the type of response attained. Thus, we can conclude that it was not likely to be a difference in tumor drug exposure that accounted for the dramatic difference in complete response rate between those patients who did not receive XRT (0/10) and those who did receive XRT (12/13; $P < 0.0001$). There are numerous reports of an additive or synergistic interaction between XRT and cisplatin [3, 4, 7]. While such an interaction may well account for this strikingly high complete response rate, a formal analysis of the nature of the interaction between XRT and cisplatin has not yet been performed. Nevertheless, the results attained with the combination of high dose intensity cisplatin and XRT are remarkable enough to merit vigorous further investigative pursuit of this program.

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