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Analysis of Tissue Platinum Distribution in Patients with Cancer of the Oesophagus

V. Troger, E. Francois, M. Frenay, M. Namer and G. Milano

The objective of this work has been to analyse the repartition of platinum (Pt) tissular levels within the tumour (T), the peritumoral adjacent non-tumoral area (P) and distant healthy tissue of the same anatomical zone (H) in oesophagus cancer. Forty-two biopsies (≃ 5 mg) have been performed under endoscopy and after informed consent in 11 patients (mean age 61 yr, range 43–74) with squamous cell carcinoma of the oesophagus treated by the neoadjuvant chemotherapy protocol including cisplatin (100 mg/m²) and 5-FU (1 g/m² × 5 days). Biopsies were done 34–36 h after cisplatin. Additional biopsies were obtained for histological controls. Pt was measured by flameless atomic absorption spectrometry. Considering Pt concentration in T, P and H there was no significant accumulation during repeated treatment (3 cycles). For all cycles, mean [S.D.] values (μg/g dry tissue) were 2.03 [2.39] for H, 2.75 [2.03] for P and 3.73 [2.3] for T (H vs. T, $P = 0.006$). In addition, Pt concentrations were found comparable between the upper and lower poles of the tumours (5 patients). Pt concentrations in T did not predict antitumour activity. These data complete the rather limited knowledge on tissular Pt levels in treated patients and suggest a decreasing gradient of Pt concentrations from tumour to healthy tissue in oesophagus cancer.
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INTRODUCTION

CISPLATIN (CDDP) is currently one of the most widely used chemotherapeutic agents. In addition to being used as a first line drug for testicular and ovarian cancers, cisplatin is given in combination with other cytostatics for other malignancies, including head and neck [1] and oesophageal cancers [2]. To date, the experimental data provided by pharmacokinetic and cellular pharmacological investigations strongly suggest that intratumoral platinum (Pt) is a determining factor for cisplatin activity. For example, animal studies have demonstrated that tissue Pt uptake is rapid, leading to persistent cellular concentrations higher than those in plasma [3–5]. In their pharmacokinetic study, Crom *et al.* [6] found that the transfer constant K12 (central to peripheral compartment) was six times higher than K21 (peripheral to central compartment). The physicochemical properties of cisplatin make this drug particularly reactive within the intracellular space, where the Cl concentration is 25 times lower than in plasma [7]. Nuclear DNA is the main, but not the sole, cellular target for cisplatin; the two molecular species form adducts which have been particularly well elucidated [8].

Numerous clinical pharmacokinetic studies have been performed on cisplatin in plasma [9]. In comparison, except for several experimental studies [3–5, 10, 11], current knowledge on tissue Pt concentrations in treated patients remains very limited [12–14]. Oesophageal cancers exhibit an interesting response rate to the cisplatin plus 5-fluorouracil (5-FU) [2]. Furthermore, these tumours lend themselves to biopsies under endoscopy.

In the present study, Pt concentrations were measured in 42 tissue samples obtained from 11 patients with oesophageal cancer treated by cisplatin–5-FU. Biopsies were taken in the tumours themselves, in the peritumoral mucosa, and at a distance, in non-tumoral healthy zones of the same anatomic site. Whenever posssible, biopsies were repeated during each treatment cycle.

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MATERIAL AND METHODS

The study population consisted of 11 patients, all male, mean age 61 years (range 43–74), with histologically confirmed squamous cell carcinoma of the oesophagus. Distribution according to the TNM classification was: three T1 lesions, seven T2 lesions and one T3 lesion. For all patients, first-line therapy consisted in a chemotherapy protocol associating cisplatin and 5-FU. Treatment was as follows: day 0, 6 h hydration with 5% dextrose (2 litres), NaCl (6 g/l) and KCl (3 g/l), followed by cisplatin (100 mg/m^2) 1 mg/min intravenously in normal saline (0.5 l) with 1.6% mannitol (0.25 l), and then 5% dextrose (1 l), NaCl (6 g/l) and KCl (3 g/l); days 1–5, 5-FU 1000 mg/m^2/24 h by continuous intravenous infusion with a controlled flow pump. Before starting the treatment, all patients had normal renal function (blood creatinine < 150 μmol/l), a Karnofsky performance status of at least 60%, and a life expectancy of at least 12 weeks. The scheduled protocol called for three courses per patient every three weeks. Response was evaluated two weeks after completion of the last chemotherapy course. Clinical response was defined using the product of two perpendicular lesion diameters. Complete response (CR) corresponded to disappearance of all clinically visible or palpable lesions; partial response (PR) was defined as tumour regression of over 50%; no response (NR) corresponded to tumour regression of 50% or less, stable disease, or progressive disease. After having obtained the patient's informed consent, biopsies (average 5 mg) were taken whenever possible from three different areas: two opposite points in the accessible tumour mass (lower and/or upper pole of the tumour), the non-malignant peritumoral mucosa (1–3 mm from the lesion), and at distance, in healthy tissue in the same anatomic zone (5–10 cm from the lesion). Biopsies were performed under endoscopy (Olympus GIF XQ 10, Tokyo), 34–36 h after administration of the cisplatin dose. Duplicate biopsies were obtained systematically for histologic examination. Excess blood was removed, and the tissue sample was placed in a screw-top plastic tube that was stored at −20°C until analysis. Biopsy material was weighed (dry weight), digested overnight by 500 μl 50% nitric acid, then homogenised in a glass–glass potter. The homogenate was dried under a nitrogen stream (50°C) and the dried residue was dissolved in 100 μl H_2O. A blood sample (3 ml) was drawn concomitantly to the biopsies. Blood samples were obtained in ethylenediaminetetraacetic acid (EDTA) tubes which were immediately placed in a water bath containing ice for transportation to the laboratory (within 10–15 min) and then centrifuged at 4°C. A 500 μl aliquot of the resulting plasma was centrifuged for 30 min at 2000 *g* and 4°C in a Centrifree micropartition unit (Amicon, Denvers, Massachusetts). The resulting ultrafiltrate fractions were used to measure filterable Pt. Total Pt was measured in the whole plasma fraction. The resulting plasma samples were stored at −20°C until analysis. Quantitative analysis of Pt in samples was performed by atomic absorption spectrophotometry (AAS) using a Perkin–Elmer model 3030 atomic absorption spectrophotometer with background correction by the Zeeman effect. Ultrafiltrates and biopsy extracts were measured without dilution. Before analysis, plasma was diluted to 1/2 using a 0.2% HNO_3 solution containing 0.01% "Triton X-100". The injected volume was 20 μl. A standard curve (0.162, 0.325 and 0.65 μg Pt/ml) was automatically plotted by an auto-sampler using a spiked blank plasma specimen that had previously been diluted to 1/2 (0.65 μg/ml). Analysis included the following steps: drying at 110°C for 40 s; washing at 1400°C for 20 s and atomisation at 2650°C with a 3 s stop flow; tube cleaning at 2650°C for 4 s. The limit of sensitivity (2 times the noise) was 5 ng/ml for plasma and 2.5 ng/ml for ultrafiltrates and biopsy specimens. Reproducibility, calculated from 25 successive series of analyses, was respectively 8.5%, 7.6% and 6.5% for Pt concentrations of 0.162, 0.325 and 0.65 μg/ml.

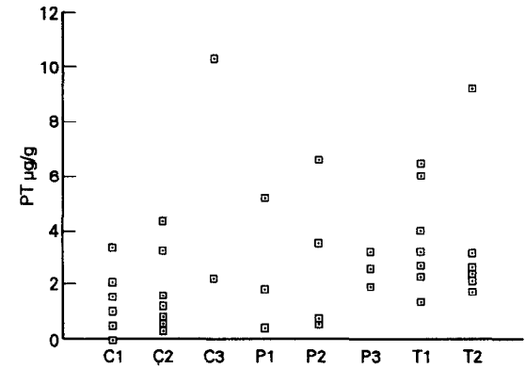

Fig. 1. Distribution of tissue platinum levels in patients with an oesophageal tumour. C = healthy distant control zone; P = healthy peritumoral zone; T = tumour; 1 = cycle no. 1; 2 = cycle no. 2; 3 = cycle no. 3. Comparisons between the various subgroups were not statistically significant except for C1 + C2 + C3 vs. T1 + T2 (P = 0.006, Mann–Whitney U test).

RESULTS

Figure 1 shows the distribution of tissue Pt concentrations for all biopsy samples. Treatment duration had no significant effect on the intracellular Pt concentration in the healthy control tissue, peritumoral areas, or tumoral zones. Considering all biopsies in each tissue subgroup, the mean (S.D.) values (μg/g dry tissue) for healthy tissue (n = 18), peritumoral areas (n = 10) and tumoral zones (n = 14) were respectively 2.03 (2.39), 2.75 (2.03) and 3.73 (2.3). There was a significant difference between the Pt concentrations in healthy tissue and in tumours (P = 0.006).

Comparison of the Pt concentrations in the lower and upper poles of the tumour in 5 patients revealed relative intratumoral homogeneity in the Pt distribution (Table 1).

Plasma Pt concentrations ranged between 0.2 and 2.68 μg/ml for total Pt, and between 0.04 and 0.075 μg/ml for ultrafilterable Pt. For paired samples, there was no statistical evidence of a relationship between the Pt concentration in plasma (total or ultrafilterable) and Pt in tissue (healthy mucosa, peritumoral area, or tumour). Individual paired data on tumoral response to

Table 1. *Individual distributions of Pt levels in oesophageal tumours*

	Platinum concentrations (μg/g dry tissue)	
Patient	Upper pole	Lower pole
1	2.80	4.12
2	1.92	2.63
3	6.16	6.59
4	1.45	2.44
5	2.30	1.81
Mean	2.93	3.51
S.D.	1.87	1.91

Wilcoxon matched-pairs signed-rank test, P = 0.106.

treatment and the corresponding Pt concentrations in tumours were available for only 4 patients (3 PR and 1 NR). Pt tumour concentrations ranged between 1.45 and 6.59 μg/g for PR, and between 3.30 and 9.36 μg/g for NR; this does not suggest any tendency for a relationship between Pt tumour concentrations and treatment response.

DISCUSSION

Much of our clinical pharmacokinetic knowledge about anticancer agents is based on drug measurements in blood samples. In theory, and quite logically, a much more relevant method for evaluating pharmacokinetic-pharmacodynamic relationships would be measurement of drug concentrations at the target site, within the tumour itself. Achieving this goal can be rather complicated for antimetabolites such as 5-FU or cytarabine, which need to be activated at the cellular level to become the active species. By contrast, cisplatin is directly active by interacting with intracellular targets. Surprisingly, few reports have been published on tissue measurements of Pt in treated patients whereas numerous reports deal with pharmacokinetic investigations in blood. In the present study, tissue Pt levels were measured in small biopsies obtained from relatively accessible oesophageal tumours and adjacent healthy tisssue during endoscopy. This allowed Pt measurements in 42 biopsy samples from 11 patients treated by cisplatin–5-FU as first-line therapy.

The tumor Pt levels found in the present study agree well with the limited data in the literature. Using an intravenous dose of 100 mg/m cisplatin, similar to that given in the pressent work, Hecquet *et al.* [14] found Pt concentrations ranging between 1 and 5.9 μg/g in neoplastic cervical tissue. In head and neck cancer patients given the same intravenous dose of cisplatin, Gouyette *et al.* [13] found tumour Pt levels between 0.71 and 7.90 μg/g. In our patients, Pt concentrations were significantly higher in the tumour than in healthy tissue from the same anatomic zone: respective means (S.D.) were 2.03 (2.39) vs. 3.73 (2.3) μg/g. These results concur with experimental data obtained in rabbits bearing the VX-2 carcinoma [10], where tumour Pt concentrations have always been higher than in adjacent muscle. Interestingly, in the present study, Pt levels in the non-tumoral peripheral mucosa were intermediate [2.75 (2.03) μg/g] between those in distant healthy tissue and those in the tumours. This suggests the possible existence of a concentration gradient between nontumoral tissue and the tumour itself. Stewart *et al.* [12] investigated Pt distribution in the human central nervous system; in their small series of 4 patients, the brain Pt distribution decreased with increasing distance from an intracerebral tumour. The kinetics of tissue Pt distribution are very rapid [15, 16], and this distribution pattern might be explained by differences in tissue irrigation in favour of the tumour. This could be the case for brain tumours where the tumour vascularisation is completely different from that of the normal brain, with marked disruption of the blood–brain barrier [17]. These hypotheses necessitate additional investigation in a larger set of patients including other easy-to-biopsy tumours such as head and neck carcinomas and uterine cervical tumours.

When account was taken of the evolution of tissue Pt levels from cycle to cycle, no statistical evidence was found for progressive accumulation of Pt in any of the subgroups investigated. Only a slight tendency for a progressive increase in Pt was seen in the healthy tissue group (Fig. 1). This absence of significant Pt accumulation concurs with the report by Hecquet *et al.* [14], who found that three weeks after the first dose and just prior to the second course, the tissue Pt levels were considerably lower (average 0.3 μg/g) although still measurable. The intratumoral Pt distribution was evaluated in a limited number of patients by comparing the drug concentrations in two opposite poles of the tumour. Results indicate a relatively good concordance between the matched samples; this suggests homogeneous Pt distribution in treated oesophageal tumours. A more extensive, but hardly feasible investigation, evaluating Pt levels from the external to the central zone of the tumour [18], would, of course, provide more positive information on this point.

An attempt was made to examine the relationship between tumour response and intratumour Pt levels, but data were available for only 4 evaluable responses. This small number of cases and the fact that 5-FU was given in association with cisplatin considerably limit the validity of the conclusion. Nevertheless, tumoral Pt levels were clearly in the same range for both NR and PR lesions. This contradicts existing experimental data for ovarian cell lines, where Pt accumulation was found to be an important mechanism of chemosensitivity [19]. However, the present results are in agreement with our preceeding conclusions concerning cervical tumours [20], and with the other limited data from treated patients [4, 21].

In conclusion, this study provides additional information which increases our knowledge about Pt pharmacokinetics at the level of tissue drug concentrations. Since cisplatin is a recognised radiosensitising agent which is homogeneously and preferentially distributed in oesophageal carcinomas, this study suggests that squamous cell carcinoma of the oesophagus is a good candidate for chemoradiation protocols using cisplatin.

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Albumin-bound and Non-protein-bound Oestradiol and Testosterone in Postmenopausal Breast Disease

Sarah Pearce, Mitchell Dowsett and J. Alan McKinna

Several studies have recently reported the percentage of non-protein-bound (NPB) oestradiol (E2) to be higher in patients with breast cancer than in normal controls. Using postmenopausal volunteers, we have examined the fractional binding of E2 and testosterone (T), as well as total E2 and T, sex-hormone binding globulin (SHBG), luteinising hormone (LH) and follicle stimulating hormone (FSH), in normal women, those at risk of developing breast cancer and women with breast cancer at first diagnosis and first recurrence. No significant differences were observed in either the concentration or in the percentage of NPB E2 or T, or in any of the other hormones measured. The validity of our observations were confirmed by expected relationships between E2, T, SHBG and body mass.
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INTRODUCTION

A LARGE AMOUNT of epidemiological and experimental evidence implicates oestrogens in the aetiology of breast cancer. However, despite many varied studies the precise role of these hormones remains unknown. Total blood oestradiol (E2) levels have frequently been measured in breast cancer patients, but this may be inappropriate for assessing biological activity as oestradiol circulates extensively bound to sex-hormone binding globulin (SHBG) and albumin, leaving only a small percentage (<2%) of the steroid in the non-protein-bound (NPB) form [1]. Recently there has been much active debate as to the biological availability of these three fractions to the tissue.

In 1981, Siiteri compared the percentage NPB E2 in breast cancer patients with that of controls and found that the level was higher in the cancer patients [2]. Since then five studies [3–7] have all reported a higher percentage of NPB E2 in breast cancer patients than in controls. Two of these studies [5, 6] also showed an increase in the albumin-bound E2 fraction in the breast cancer patients. Two other studies [8, 9], found no significant difference between breast cancer patients and controls. Moore and his colleagues also reported a lower proportion of E2 bound to SHBG in the blood of women who went on to develop breast cancer than in those who did not [10]. Based on this evidence coupled with the observation that Japanese women (who have a lower incidence of breast cancer than western women) also have a higher proportion of E2 bound to SHBG [10], they proposed that this parameter may be a marker for breast cancer risk.

For a given change in SHBG binding capacity, the change in percentage NPB testosterone (T) is greater than that in the percentage NPB E2 [1]. Any difference observed in percentage of NPB E2 would therefore be expected to manifest itself in a greater change in percent NPB T. This parameter would therefore be expected to be a more sensitive marker of breast cancer risk than NPB E2. In addition if this effect were reflected in an increased concentration of NPB T, it may be important in increasing the availability of T to the aromatase enzyme, which is responsible for converting androgens into oestrogens.

In this study we have measured total, albumin-bound and

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