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Use of Intravenous Microdialysis to Monitor Changes in Serotonin Release and Metabolism Induced by Cisplatin in Cancer Patients: Comparative Effects of Granisetron and Ondansetron[1]

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ABSTRACT

Serotonin [5-hydroxytryptamine (5-HT)] is involved in the production of emesis associated with cisplatin treatment. Serotonin released from intestinal enterochromaffin cells may act either directly on vagal afferents and/or pass to the circulation and stimulate central emetic centers. However, the role for circulating 5-HT has not been determined. In this study, i.v. microdialysis probes were used to investigate 1) cisplatin-induced changes in 5-HT release and metabolism assessed through changes in blood dialysate levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA), 2) whether free 5-HT in blood increases after cisplatin, and 3) whether granisetron and ondansetron exert different effects on cisplatin-induced 5-HT release and metabolism. Control experiments conducted in 10 healthy volunteers revealed stable 5-HT and 5-HIAA dialysate levels for a period of 6 h. In patients with cancer ($n = 16$), baseline blood dialysate 5-HIAA concentrations averaged 2.98 ± 0.38 ng/ml, which were equivalent to a total of 94 ± 10 pg in the 30-min collection period at a flow rate of 1 μl/min. Cisplatin (89 ± 2.9 mg of cisplatin/m^2) produced a gradual increase in blood dialysate 5-HIAA levels (104 ± 4% increase at 4 h). Increases in dialysate 5-HIAA were associated with increases in the urinary excretion of this metabolite. After cisplatin, dialysate 5-HIAA levels increased to 5.89 ± 0.5 ng/ml in granisetron and to 5.27 ± 0.9 ng/ml in ondansetron-treated patients ($P > .1$). Similar time courses and percentages of increase in blood dialysate and urinary 5-HIAA levels were observed in ondansetron- and granisetron-treated patients. Contrary to 5-HIAA, no significant increases in dialysate 5-HT were observed from 2 to 8 h after cisplatin either for the total group or for each of the groups separately. In conclusion, i.v. microdialysis probes coupled to HPLC-EC allowed the continuos monitoring of free-5-HT and 5-HIAA in blood. Cisplatin-induced increases in blood 5-HIAA were not associated with increases in 5-HT blood dialysates. These results argue against a possible action of free 5-HT in plasma on the chemoreceptor trigger zone (unprotected from the blood brain barrier) but support the view that 5-HT released within the intestinal wall triggers emesis after cisplatin. Our results argue against the view that at clinically effective doses, granisetron and ondansetron exert different actions on cisplatin-induced 5-HT release and metabolism.

Serotonin [5-hydroxytryptamine (5-HT)] plays a fundamental role in the production of nausea and emesis associated with cancer chemotherapy drug regimens (Cubeddu et al., 1990; Andrews, 1994; Alfieri and Cubeddu, 1995; Andrews and Davis, 1995). Cytotoxic drugs have been shown to induce the release of 5-HT from enterochromaffin cells (Schwörer et al., 1991; Fukui et al., 1993; Minami et al., 1997). In patients with cancer, the acute vomiting produced by cisplatin is paralleled by increases in the urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) and of plasma chromogranin A, markers of enterochromaffin cell activity (Cubeddu et al., 1990; 1995b,c). Once released, 5-HT triggers vomiting by stimulating 5-HT$_3$ receptors located in vagal afferent nerves and/or in central areas (chemoreceptor-trigger zone) (Hawthorn et al., 1988; Carl et al., 1989; Andrews, 1994). There is controversy on whether the circulating levels of free-5-HT increase after treatment with cytotoxic drugs (Barnes et al., 1990; Cubeddu et al., 1992; Fukui et al., 1993). Part of the discrepancies may be related to the methodology used for the collection and preparation of the platelet-free plasma (Cubeddu et al., 1995a). The application of i.v. microdialysis techniques may provide an answer to whether free-blood 5-HT increases after cisplatin. These techniques allow

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); 5-HIAA, 5-hydroxyindoleacetic acid.

the continuous monitoring of blood concentrations of analytes and circumvent problems related to sample handling (i.e., 5-HT uptake into platelets during sample processing).

Selective antagonists of 5-HT_3 receptors are key drugs in the management of nausea and vomiting associated with cancer chemotherapeutic drugs (Costall et al., 1986; Miner and Sanger, 1986; Cubeddu et al., 1990). Ondansetron, granisetron, and others have been shown to reduce the incidence of nausea and emesis associated with radiation therapy and cisplatin- and cyclophosphamide-based chemotherapies (Cunningham 1997; Roila et al., 1997; Perez, 1998; Poon and Chow, 1998). Although these agents block 5-HT_3 receptors, recent studies have indicated that ondansetron and granisetron have additional actions. Granisetron showed noncompetitive kinetics at a 5-HT_3 receptor assay, at which ondansetron behaved as a competitive antagonist (Blower, 1995; Ito et al., 1995). In addition, by using a test for visceral hypersensitivity in rats, Langlois et al. (1996) demonstrated that granisetron, but not ondansetron, inhibited the effects of gut distention and chemical irritation. In in vitro studies, granisetron has been shown to inhibit the release of 5-HT from enterochromaffin cells (Minami et al., 1995, 1997), whereas ondansetron failed to inhibit the increases in urinary of 5-HIAA and in plasma chromogranin A induced by cisplatin in patients with cancer (Cubeddu et al., 1990, 1995b). However, no study has assessed the effects of ondansetron and granisetron on cisplatin-induced 5-HT release and metabolism in vivo and in patients.

In this study, we used i.v. microdialysis probes to monitor the changes in blood 5-HIAA and 5-HT in patients with cancer receiving cisplatin-based chemotherapy to determine 1) the applicability of i.v. microdialysis techniques for the monitoring and quantification of cisplatin-induced changes in 5-HT release and metabolism, 2) whether free 5-HT in blood increases at a time at which intense emesis has been reported to occur after treatment with cisplatin, and 3) whether granisetron and ondansetron have different effects on 5-HT release and metabolism, by monitoring the increases in blood 5-HT and 5-HIAA and in urinary 5-HIAA, induced by cisplatin, in patients treated with one or the other selective 5-HT_3 antagonists. Additional experiments were performed in untreated volunteers to assess the variability and reproducibility of blood 5-HT and 5-HIAA dialysate levels with our probes in subjects not treated with cisplatin.

Materials and Methods

Patients. Ten healthy volunteers (36 ± 2 years, eight men and two women, 71.2 ± 3 kg) were studied to assess the range of basal levels, the presence of temporal changes, and the reproducibility of blood 5-HT and 5-HIAA dialysate concentrations in subjects not treated with cisplatin. A written informed consent was obtained each subject. In addition, a total of 16 consecutive hospitalized patients (18 years old or older) with histologically confirmed cancer and scheduled to receive moderate- to high-dose cisplatin-based regimens (≥50 mg/m^2) were enrolled in the study. The following inclusion criteria were used: diagnosis of cancer, age of 19 to 60 years, Karnosfky index of ≥80%, and treatment with cisplatin at doses of ≥50 mg/m^2. Cisplatin was dissolved in 500 ml of 5% dextrose in 0.9% sodium chloride and administered as a 60-min i.v. infusion. Patients were continuously hydrated with 5% dextrose at a rate of 200 ml/h, starting 2 h before the session and terminating at the end of the dialysis session. The primary agent (cisplatin) was followed by the administration of other chemotherapeutic drugs as required for treatment of the patient's neoplasia. Other chemotherapies included methotrexate, 5-fluorouracil, doxorubicin, and gemzar (Table 1). Subjects did not ingest any food immediately before and during the experiment. In addition, to further prevent acute gastrointestinal toxicity (nausea, retching, and vomiting), all subjects received effective i.v. antiemetic treatment 30 min before the initiation of the cisplatin infusion. Cancer patients were randomized to two groups of eight patients each: one group received antiemetic treatment with 8 mg of ondansetron and 8 mg of dexamethasone, and the other received 2 mg of granisetron plus 8 mg of dexamethasone. The glucocorticoid was added to maximize the control of emesis (Roila et al., 1997; Perez, 1998). All measurements were made the day of the cisplatin treatment (first day of chemotherapy). Written informed consent was obtained from each patient, and the protocols were evaluated and approved by the Institutional Review Board of the Luis Razetti Oncology Hospital (Caracas, Venezuela).

Intravenous Microdialysis Probes. The flexible removable probes were constructed at the Laboratory of Behavioral Physiology of the Universidad de los Andes (Merida, Venezuela) and validated as described in detail by Paez and Hernandez (1996, 1997). The active dialysis area of the probes consisted of a 20-mm-long cellulose hollow fiber with a 200-μm outside diameter and a 13,000 mol. wt. cutoff size (Spectrum Medical Industries, Los Angeles, CA). The probes and connections of polyethylene were individually packed and sterilized with ethylene oxide for 12 h and ventilated for 24 h before use. The time response of the probe was less than minutes. The calculated dead end volume for the probe was 2.1 μl, which represented 7% of the volume of the collected sample in the present experiments. The dead volume from the probe to the collection tube was 20 μl; because the flow rate was 1 μl/min, the delay between the changes in plasma and the changes recorded by us was 20 min. The in vivo recovery for 5-HT and 5-HIAA averaged 98.6% (Paez and Hernandez, 1998).

Dialysis Session. A similar procedure was used for healthy subjects and patients with cancer. A 1-inch long, 22-gauge sterile Teflon catheter (Jelco; Dupont) was placed in one antebrachial vein of the arm not receiving chemotherapeutic drugs. The i.v. dialysis probe was inserted into the venous catheter, and the entire 20-mm-long cellulose hollow fiber passed through and protruded into the bloodstream (active dialysis area). The dialysis probe was connected to a syringe pump and continuously perfused with 0.9% sterile saline at a rate of 1 μl/min. A 150-min period of equilibration was used to minimize any possibility of contamination of plasma dialysates with any 5-HT that might have been released from platelets during the probe insertion. Subsequently, the dialysis probe outflow was collected into polypropylene tubes, placed on dry ice, and subsequently stored at −60°C until assayed. Thirty-minute samples were collected throughout the experiment. All samples were assayed within 24 h of collection.

TABLE 1
Patient characteristics
Ages and weights are given as mean ± S.E.

Age (y)	52.7 ± 2.8
Sex (M/F)	13/2
Weight (kg)	70.7 ± 4.3
Type of tumor (*n*)	
Head and neck	12
Osteosarcoma	1
Lung	1
Penis	2
Chemotherapeutic drugs (*n*)	
Cisplatin	16
Cisplatin alone	0
5-Fluorouracil	14
Gemzar	1
Doxorubicin	1

Analytical Methods. All samples were assayed within 24 h of collection. 5-HT and 5-HIAA were quantified by means of HPLC with an amperometric electrochemical detection (model 464; Waters). The detector potential (oxidation potential) was set at +710 mV between a glassy carbon working electrode and an Ag/AgCl reference electrode. A 20-μl aliquot of each sample dialysate was injected into the HPLC system. Separation of the compounds was achieved by a means of a 3-μm Waters Novapack C18 (3.9 × 150 mm) reversed phase column. The mobile phase consisted of 0.1 mM citric acid, 0.1 mM formic acid, acetonitrile (9 v/v), 1 mM disodium EDTA, and 0.08 mM octanesulfonic acid. The pH of the mobile phase was adjusted to 3.4 with potassium hydroxide. The mobile phase was filtered and degassed and delivered with a dual piston pump (model 510; Waters) at a constant flow rate of 0.7 ml/min. Peaks were captured in and analyzed by a Waters Data Module (model 740). Separation of interfering peaks was achieved on an individual basis by adjusting the pH and the acetonitrile concentration of the mobile phase. Identification on the basis of retention times and measurements of the compounds by peak heights in the samples were achieved by comparisons with 5-HT and 5-HIAA external standards. The standards were prepared as 1 mg/ml stock solution in 0.1 M HCl with 100 μM disodium EDTA. Linearity was determined by injecting increasing concentrations of 5-HT and 5-HIAA (10–400 pg/20 μl). The detection limit was established as the mass of 5-HT or 5-HIAA that generated a signal three times as large as noise. For 5-HT, the detection signal was 10 pg, and its average retention time of 8.4 min. For 5-HIAA, the assay sensitivity was 10 pg, and the average retention time was 5.7 min. The intra-assay and interassay variations for 5-HT and 5-HIAA were 5.9 to 6.5% (S.D.) and 8.2 to 10.3%, respectively. .

Urinary 5-HIAA. On the day of chemotherapy, urine was collected for 24 h starting with the infusion of cisplatin (time 0). Four consecutive 2-h samples were obtained (0–2, 2–4, 4–6, and 6–8), followed by a 16-h sample to complete the 24-h collection period. Urine samples were acidified with $HClO_4$ (0.1 M final concentration), centrifuged, and diluted 1:50 in mobile phase before quantification. A 20-μl aliquot of the acidified, diluted urine was injected (loop injection) into the injection port. Urinary 5-HIAA was quantified with an HPLC procedure with electrochemical detection as described above.

Statistical Analysis. Comparisons between baseline values and values obtained at different times after administration of cisplatin was achieved with ANCOVA because each patient provided basal and post-treatment values. Comparisons between ondansetron and granisetron were evaluated by two-way ANOVA, followed by Duncan's test. Results were expressed either as ng/ml dialysate, pg/30-min sample, or percentage of change from baseline levels. Urinary excretion of 5-HIAA was expressed as μg/2 h. Results were expressed as mean ± S.E. for n observations. Significance was set at $P < .05$.

Results

The demographic characteristics of the patients with cancer are shown in Table 1. Most patients had solid tumors of head and neck. The mean dose of cisplatin administered to the patients was 89 ± 2.9 mg/m^2. With the antiemetic protection provided, none of the patients experienced any vomiting during the period of microdialysis. For the patients with cancer, the duration of the microdialysis session averaged 9.3± 0.5 h. After 150 min of stabilization, the 30-min sample collection began. The time course for the changes in dialysate 5-HIAA and 5-HT levels is shown in Fig. 1. Baseline 5-HIAA averaged 2.98 ± 0.38 ng/ml, which corresponded to 94 ± 10 pg/30 min (95% confidence interval, 72–116 pg/30 min). 5-HIAA showed a gradual increase, reaching its maximum (104 ± 4% above baseline levels) from 3.5 to 6 h after cisplatin. Similarly to blood dialysate levels, the urinary excretion rate of 5-HIAA peaked at 4 to 6 h after cisplatin, returning to baseline levels in the 8- to 24-h sample (Table 2). Higher dialysate levels were associated with higher urinary excretion rates.

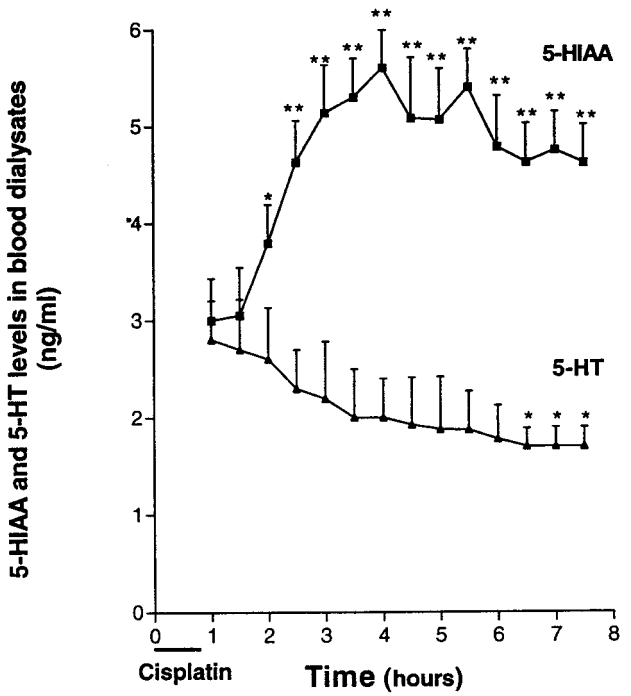

Fig. 1. Blood dialysate levels of 5-HT and 5-HIAA in patients with cancer: effects of cisplatin. A microdialysis probe was inserted in an antebrachial vein. 5-HT and 5-HIAA concentrations (ng/ml) were measured in the dialysate (30-min collection samples). Abscissa, time in hours after cisplatin and period of cisplatin infusion (solid bar). Shown are mean ± S.E. values (n = 16). *$P < .05$ and **$P < .01$, significantly different from baseline.

TABLE 2

Urinary excretion of 5-HIAA in patients with cancer treated with cisplatin

Urines were collected for 24 h, beginning with the infusion of cisplatin (time 0). Four consecutive 2-h samples were obtained (0–2, 2–4, 4–6, and 4–8), followed by a 16-h sample to complete the 24-h collection period. Shown are mean ± S.E. values for the urinary excretion of 5-HIAA expressed as μg/2 h.

Time Period				
0–2 h	2–4 h	4–6 h	6–8 h	8–24 h
264 ± 40	604 ± 129 ($P < .05$)	828 ± 194 ($P < .01$)	607 ± 60 ($P < .01$)	253 ± 34 ($P < .05$)

Baseline dialysate 5-HIAA averaged 3.26± 0.98 ng/ml in the granisetron group and 2.84 ± 0.57 ng/ml in the ondansetron group ($P > .1$; Fig. 2). Cisplatin induced similar quantitative and qualitative changes in blood dialysate 5-HIAA levels in ondansetron- and granisetron-treated patients. For granisetron-treated patients, peak levels averaged 5.89 ± 0.49 ng/ml, and for ondansetron-treated patients, peak levels were 5.27 ± 0.12 ng/ml ($P > .1$). No significant differences were observed for the magnitude and the time course of the increase in dialysate 5-HIAA between granisetron- and ondansetron-treated patients (Fig. 2). The urinary excretion of 5-HIAA increased after cisplatin administration in both ondansetron- and granisetron-treated groups (Fig. 3). Peak urinary excretion rates (μg/2 h) were observed for both groups from 4 to 6 h after cisplatin. No significant differences for the increases in urinary 5-HIAA induced by cisplatin were observed between ondansetron- and

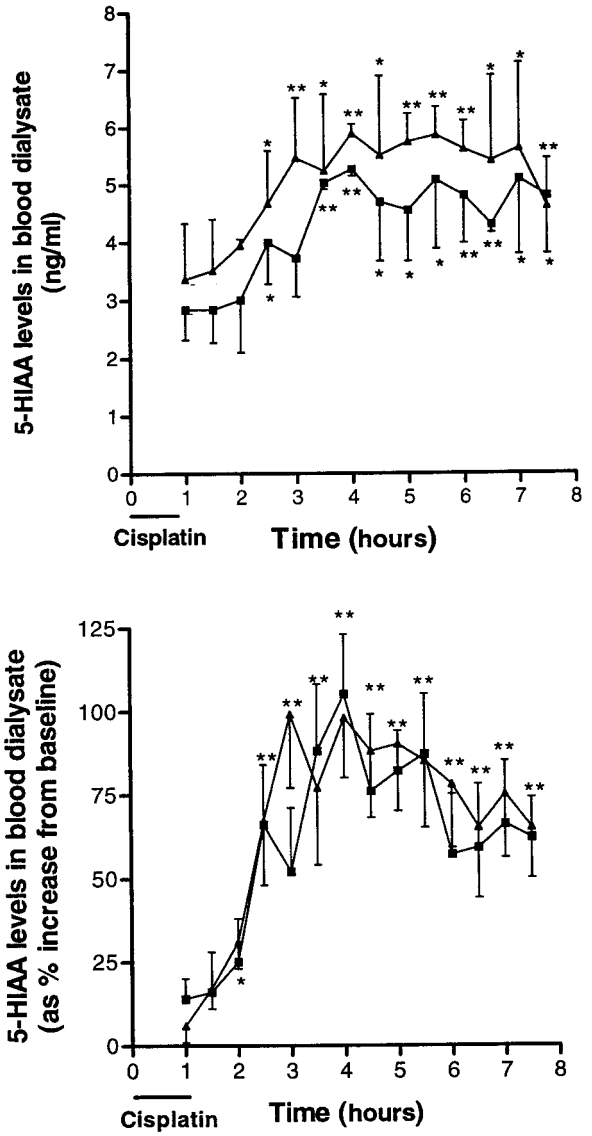

Fig. 2. Comparative effects of ondansetron and granisetron on cisplatin-induced increases in 5-HIAA levels in blood dialysates in patients with cancer. A microdialysis probe was inserted in an antebrachial vein, and 5-HIAA concentrations were measured in the dialysate (30-min collection samples) before and after cisplatin administration. Granisetron (▲; 2 mg) combined with 8 mg of dexamethasone or ondansetron (■; 8 mg) combined with 8 mg of dexamethasone was administered i.v. 30 min before the cisplatin. Top, 5-HIAA concentrations (ng/ml) in blood dialysates. Bottom, percentage of increase above baseline in 5-HIAA concentrations in blood dialysates. Abscissa, time in hours after cisplatin and period of cisplatin infusion (solid bar). Shown are mean ± S.E. values (n = 8 for granisetron and n = 8 for ondansetron). *$P < .05$ and **$P < .01$, significantly different from baseline. Bottom, significances were only displayed once because similar P values were obtained for both groups.

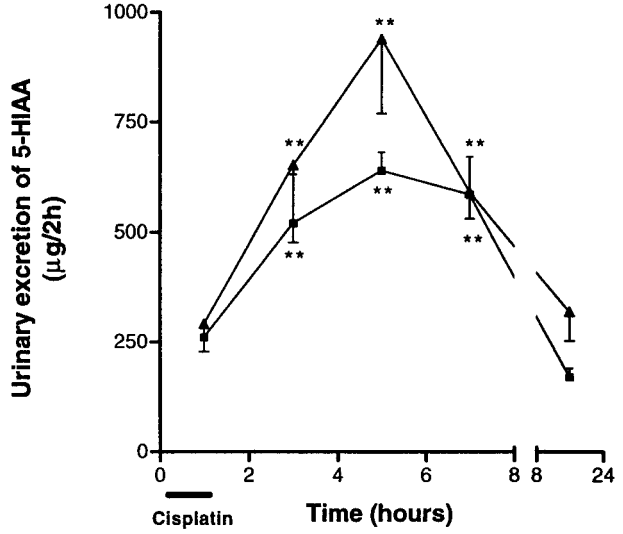

Fig. 3. Comparative effects of ondansetron and granisetron on cisplatin-induced increases in the urinary excretion of 5-HIAA. On the day of chemotherapy, urine samples were collected for 24 h starting with the infusion of cisplatin (time 0). Four consecutive 2-h samples were obtained (0–2, 2–4, 4–6, and 6–8), followed by a 16-h sample to complete the 24-h collection period. Granisetron (▲; 2 mg) combined with 8 mg of dexamethasone or ondansetron (■; 8 mg) combined with 8 mg of dexamethasone was administered i.v. 30 min before the cisplatin. Shown are mean ± S.E. values (n = 8 for ondansetron and n = 8 for granisetron).**$P < .01$, significantly different from baseline.

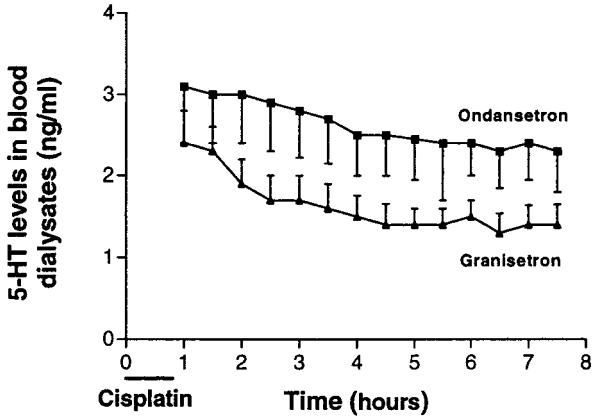

Fig. 4. 5-HT concentrations in blood dialysates of patients with cancer treated with cisplatin: effects of ondansetron and granisetron. A microdialysis probe was inserted in an antebrachial vein. 5-HT and 5-HIAA concentrations (ng/ml) were measured in the dialysate (30-min collection samples). Granisetron (▲; 2 mg) combined with 8 mg of dexamethasone or ondansetron (■; 8 mg) combined with 8 mg of dexamethasone was administered i.v. 30 min before the cisplatin. Abscissa, time in hours after cisplatin and period of cisplatin infusion (solid bar). Shown are mean ± S.E. values (n = for ondansetron and n = 8 for granisetron).

granisetron-treated patients (Fig. 3).

Contrary to 5-HIAA, dialysate 5-HT showed a gradual decrease throughout the experiment (Fig. 1). From 2 to 7 h after cisplatin, dialysate 5-HT levels averaged 2.1 ± 0.1 ng/ml, and no significant increases were observed during this period of observation, despite marked increases in dialysate 5-HIAA concentrations. Although the baseline concentrations of 5-HT in the blood dialysates were 25% greater in ondansetron- than in granisetron-treated patients, no increases in blood 5-HT dialysate levels were observed in either group of patients after cisplatin treatment (Fig. 4).

Blood dialysate 5-HIAA and 5-HT concentrations were measured in healthy volunteers (Table 3). The duration of the microdialysis session was 8 h. After 150 min of stabilization, the 30-min sample collection began. Baseline values ranged from 0.55 to 4.6 ng/ml for 5-HT and from 0.70 to 3.5 ng/ml for 5-HIAA. No significant increases or decreases in the dialysate concentrations of 5-HT and 5-HIAA were observed during the period of collection (Table 3). No significant differences in the concentrations of 5-HIAA and 5-HT in dialysates were encountered between patients with cancer and healthy volunteers.

TABLE 3

Blood dialysate 5-HT and 5-HIAA concentrations in healthy volunteers

A microdialysis probe was inserted in an antebrachial vein. After 150 min of stabilization, 5-HT and 5-HIAA concentrations were measured in the dialysates for a period of 6 h. Shown are mean ± S.E. values (n = 10). For 5-HT and 5-HIAA, no significant differences were observed between basal levels and levels at 1, 2, 3, 4, 5, and 6 h.

	Time						
	0 h	1 h	2 h	3 h	4 h	5 h	6 h
				ng/ml			
5-HT	1.9 ± 0.5	1.4 ± 0.4	1.9 ± 0.6	2.1 ± 0.6	1.9 ± 0.4	1.5 ± 0.3	1.4 ± 0.4
5-HIAA	1.9 ± 0.3	2.2 ± 0.3	2.2 ± 0.3	2.0 ± 0.3	2.1 ± 0.3	2.1 ± 0.3	2.1 ± 0.4

Discussion

Previous studies have shown the advantages of i.v. microdialysis for the continuous monitoring of endogenous compounds. Our microdialysis probes have been shown to have a quick response time and high and reproducible in vitro and in vivo recoveries for substances such as glucose, 5-HT, and 5-HIAA (Paez and Hernandez, 1996, 1997; present study). Due to the very high recoveries, the 5-HIAA and 5-HT concentrations in the dialysates were comparable to those described for the normal range of both substances in plasma (Anderson et al., 1987; Cubeddu et al., 1992; Lechin et al., 1996). The i.v. microdialysis probes used detected large increases in blood dialysate 5-HIAA levels after cisplatin administration, supporting the view that cisplatin induces the release of 5-HT. The increases in blood dialysate 5-HIAA were associated with increases in the urinary excretion of this metabolite (present study). The increases in 5-HIAA are due to the cisplatin and not to any additional treatment or factor because 1) no changes in blood dialysate 5-HIAA levels were observed in untreated healthy volunteers (present study), 2) the urinary excretion of 5-HIAA (μg/2 h or μg/mg creatinine) does not increase throughout a period of 10 h in volunteers exposed to a diuretic/hydration protocol similar to that received by patients with cancer (Cubeddu et al., 1990), 3) cisplatin increases 5-HIAA excretion and plasma chromogranin A in the absence of antiemetic treatment (Cubeddu et al., 1992, 1995a), and 4) no increases in 5-HIAA excretion and of plasma chromogranin A occur in patients treated with cyclophosphamide (not cisplatin) who received ondansetron as prophylactic antiemetic (Cubeddu et al., 1992, 1995c).

The increases in dialysate and in urinary 5-HIAA occurred at times (2–6 h after cisplatin) at which intense vomiting is known to develop after treatment with cisplatin (Cubeddu et al., 1990; Cubeddu and Hoffmann, 1994). The increases in blood dialysate 5-HIAA were not accompanied with increases in dialysate 5-HT (present study). With the use of repeated venipuncture (or blood draws from the same catheter), anticoagulant addition (sodium citrate), and sample processing (centrifugation for the separation of plasma from cells), other two studies failed to report increases in plasma 5-HT after cisplatin or cyclophosphamide treatments (Barnes et al., 1990; Cubeddu et al., 1992). Because 5-HT is avidly and effectively taken up by platelets, its rapid uptake into platelets may account for the failure to observe increases in blood dialysate levels. However, this is unlikely because the content and concentration of 5-HT in platelets were not increased after treatment with cisplatin, despite large increases in the urinary excretion of 5-HIAA (1.5–2 mg in 4 h; Cubeddu, 1992; Cubeddu et al., 1992). In conclusion, our findings support the view that free 5-HT in plasma does not increase at times at which cisplatin is known to produce intense emesis and at which it induces large increases in dialysate and urinary 5-HIAA levels. Previous studies have shown that cisplatin-induced increases in 5-HIAA were paralleled by increases in plasma chromogranin A concentrations (Cubeddu et al., 1995b,c). It is known that in the absence of endocrine tumors, the increases in 5-HIAA and chromogranin A levels represent 5-HT release from the gastrointestinal tract (Bertaccini, 1960; Bertaccini and Chieppa, 1960; Bargsten and Grube, 1992; Cubeddu et al., 1995a). Therefore, these results, as well as other obtained in experimental animals (Hawthorn et al., 1988; Schwörer et al., 1991; Fukui et al., 1993; Andrews, 1994), suggest that cisplatin releases 5-HT from gastrointestinal stores (enterochromaffin cells). The 5-HT released within the gut would stimulate 5-HT_3 receptors located in vagal afferents inducing marked increases in visceral afferent inputs to the emetic centers, leading to nausea and emesis (for reviews, see Andrews and Davis, 1995; Andrews et al., 1998). Accordingly, the majority of the 5-HT released from the enterochromaffin cells would be metabolized to 5-HIAA within the intestinal wall and/or during its passage through the liver, leading to large increases in plasma and urinary 5-HIAA not associated with increases in plasma 5-HT. The results of this study support the view that circulating 5-HT does not play a role in the emetic response associated with cisplatin treatment.

Selective 5-HT_3 receptor antagonists are key drugs for the management of nausea and vomiting associated with cancer chemotherapeutic drugs (Costall et al., 1986; Miner and Sanger, 1986; Cubeddu et al., 1990; Andrews, 1994; Cunningham, 1997). Recent findings suggest the existence of differences among 5-HT_3 antagonists (Blower, 1995; Ito et al., 1995; Langlois et al., 1996). Granisetron has been reported to inhibit the release of 5-HT from enterochromaffin cells (Minami et al., 1995, 1997), whereas ondansetron failed to inhibit the increases in urinary 5-HIAA and in plasma chromogranin A induced by cisplatin in patients with cancer (Cubeddu et al., 1990, 1995b). It has been proposed that release-facilitatory 5-HT_3 autoreceptors on enterochromaffin cells could differ from the 5-HT_3 receptors located on vagal afferent fibers (Gebauer et al., 1993; Minami et al., 1995; Schwörer and Ramadori, 1998) and that granisetron would be effective in blocking both receptors. The blockade of facilitatory 5-HT_3 autoreceptors would be expected to reduce 5-HT release. However, our findings in patients with cancer argue against the existence of differences between ondansetron and granisetron on cisplatin-induced 5-HT release, because comparable increases in blood dialysate and urinary 5-HIAA levels were produced by cisplatin in ondansetron- and granisetron-treated subjects. Previous studies have shown that ondansetron neither inhibits nor enhances cisplatin-induced increases in urinary 5-HIAA and in plasma chromogranin A

in patients with cancer, an effect concordant with its mechanism of action (i.e., antagonism of 5-HT_3 receptors; Cubeddu et al., 1992, 1995a; Andrews et al., 1998). Although we cannot rule out other sites of action, our findings favor the view that similar to ondansetron, granisetron prevents emesis mainly by blocking the effects of 5-HT at 5-HT_3 receptors on vagal afferents and does not seem to exert an additional inhibitory effect on 5-HT release.

For ethical reasons, all of our study patients received preventive antiemetic therapy. In this study, dexamethasone was used in combination with the 5-HT_3 antagonist to maximize emetic control. The addition of dexamethasone to granisetron or ondansetron improves the control of emesis in patients treated with moderately to highly emetogenic chemotherapies (Roila et al., 1997; Perez, 1998). The possibility that dexamethasone would affect the release of 5-HT induced by cisplatin is improbable because the increases in urinary 5-HIAA induced by cisplatin were not affected by dexamethasone at doses 2.5 times higher than those used in this study (Cubeddu and Hoffmann, 1993, 1994). In addition, similar increases in urinary 5-HIAA and in plasma chromogranin A were observed in patients treated with dexamethasone, metoclopramide, ondansetron, or placebo (Cubeddu and Hoffmann, 1993; Cubeddu et al., 1995a). Furthermore, cisplatin did not increase plasma-free and platelet 5-HT levels in patients receiving placebo antiemetic or ondansetron as the sole antiemetic (no dexamethasone; Cubeddu et al., 1992). Therefore, the use of dexamethasone should not be the reason for the similar effects of granisetron and ondansetron on blood dialysate 5-HIAA concentrations and for the lack of increases in dialysate 5-HT after cisplatin.

In conclusion, i.v. microdialysis probes coupled to HPLC-EC allowed the continuous monitoring of free-5-HT and 5-HIAA levels in the blood of healthy volunteers and of patients with cancer. The increases in 5-HIAA levels were associated with increases in urinary 5-HIAA, and these increases occurred at times at which acute emesis is known to develop after cisplatin (Cubeddu et al., 1990, 1995a). The increases in free 5-HIAA were not associated with increases in free 5-HT. These results argue against the role of a central action of circulating 5-HT on the chemoreceptor trigger zone (unprotected from the blood-brain barrier) but rather support the view that the free 5-HT within the intestinal wall is involved in triggering the emetic response after cisplatin chemotherapy. The increases in circulating 5-HIAA levels represent the 5-HT metabolized either within the gut or on its passage through the liver after being released from the gut. Our results also indicate that at clinically effective doses, granisetron and ondansetron do not differently affect the magnitude and time course of 5-HT release induced by cisplatin.

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