Improved Diagnosis of Carcinoid Tumors by Measurement of Platelet Serotonin

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Carcinoid patients are diagnosed biochemically on the basis of increased urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA); urinary and platelet serotonin concentrations are considered to provide complementary information. Using established HPLC methods with fluorometric detection, we evaluated the clinical usefulness of measurements of urinary 5-HIAA and urinary, plasma, and platelet serotonin in 30 consecutive patients with histologically proven carcinoid tumors of fore-, mid-, and hindgut origin before treatment. Ten patients showed no signs of serotonin overproduction; 14 had increased concentrations of urinary 5-HIAA and platelet serotonin; and platelet serotonin, but not urinary 5-HIAA, was increased in 6. None had increased urinary 5-HIAA excretion without an increase in platelet serotonin content. In cases with high rates of tumor serotonin secretion, platelet serotonin reached a maximum and did not correlate with serotonin secretion rate, whereas urinary 5-HIAA was correlated. Increased platelet serotonin was correlated with increased plasma serotonin and with occurrence of carcinoid syndrome. Increased urinary serotonin, allegedly caused by increases in circulating 5-hydroxytryptophan, almost invariably coincided with increased platelet serotonin, but not necessarily with above-normal urinary 5-HIAA excretion. From these results and long-term monitoring of three patients during treatment, we conclude that platelet serotonin is more sensitive than urinary 5-HIAA for detecting carcinoids that secrete only small amounts of serotonin.

Additional Keyphrases: tumor markers • cancer • 5-hydroxyindoleacetic acid • urine

Carcinoid tumors are APUD-omas (characterized by amine precursor uptake and decarboxylation) that arise from enterochromaffin cells (1, 2).4 Embryologically, these cells are derived from the primitive neuronal ectoderm (3). Foregut carcinoids arise from the respiratory tract, pancreas, stomach, and duodenum; midgut carcinoids, from the ileum and appendix; and hindgut carcinoids, from the left colon and rectum (4). The tumors appear most frequently in the midgut (~70% of the cases) but also in the fore- and hindgut (~13% and ~17%, respectively). Depending on the origin and size of the primary lesion, distant metastases (usually to liver) can occur. The predilection to deliver metastases amounts to 14–50%, 2–60%, and 3% in fore-, mid-, and hindgut, respectively (5).

The occurrence and severity of the carcinoid syndrome, the endocrine manifestation of enterochromaffin cell neoplasm (6), are directly related to tumor bulk in an area that drains into the systemic circulation (1). It is characterized by cutaneous flushing, diarrhea, valvular lesions of the right side of the heart, and bronchoconstriction. In particular, midgut carcinoids with distant metastases and bronchus carcinoids are known to cause the syndrome. Depending on their tissue of origin and the presence of metastases, these tumors can cause excessive synthesis, storage, and release of peptides and biogenic amines, of which serotonin is the most prominent (7). Characteristically, carcinoid tumors arising from the midgut readily produce and secrete serotonin, whereas those from the foregut secrete both serotonin and its precursor 5-hydroxytryptophan (5-HTP), although to a lesser extent. Carcinoid tumors from hindgut usually do not secrete substantial amounts of serotonin (5). This biogenic amine with prominent vasoactive properties plays an important role in the etiology of some symptoms of the carcinoid syndrome, although other mediators are also implicated (1, 5). Released serotonin is actively taken up by platelets and various tissues (8); in platelets, it is stored in granules (9).

Biochemically, carcinoid patients are diagnosed on the basis of increased urinary excretion of the major metabolite of serotonin, 5-hydroxyindoleacetic acid (5-HIAA) (10, 11), the measurement of which is regarded as the most reliable biochemical test for a serotonin-secreting tumor (1, 10, 12, 13). Probably depending on the distribution between fore-, mid-, and hindgut carcinoids in the groups studied, the diagnostic sensitivity of this metabolite is reportedly 18–88% (14, 15). In one study, additional determinations of urinary and platelet serotonin, determined by a not readily available radioenzymatic method, moderately increased the sensitivity from 75% to 84% (12) and therefore were considered to provide complementary information.

Using an established, easily accessible method (HPLC with fluorometric detection), we evaluated the usefulness of measurements of 5-HIAA and serotonin concentrations in urine and of platelet serotonin content in 30 consecutive patients with histologically proven carcinoid tumors of various origins. The effects of tumor load reduction and relapse on the platelet serotonin content and urinary 5-HIAA excretion rate are illustrated by a longitudinal study of three patients.

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4 Nonstandard abbreviations: APUD, amine precursor uptake and decarboxylation; 5-HTP, 5-hydroxytryptophan; and 5-HIAA, 5-hydroxyindoleacetic acid.

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Subjects and Methods

Subjects. For four years, we studied 30 consecutive patients (13 females, 17 males; median age 59.5, range 13–79 years) with histologically proven carcinoid tumors (Table 1). Twenty-seven of these patients were monitored during surgical or systemic treatment. Twenty-six healthy persons (11 women, 15 men; median age 30, range 18–48 years) served as a control group.

Samples. Blood and urine were sampled without dietary restrictions and in an undefined metabolic state. We collected 24-h urine samples from patients and controls in 2-L brown polypropylene bottles (Sarstedt, Nuembrecht, F.R.G.) containing ~250 mg each of Na₂S₂O₅ and EDTA as preservatives. Samples were acidified to pH 4 with acetic acid before freezing. Venous blood samples were collected in 10-mL Vacutainer Tubes (Becton-Dickinson, Meylan Cedex, France) containing 0.12 mL of 0.34 mol/L EDTA solution and put on ice without delay. Platelet-rich plasma was prepared from whole blood within 1 h after sampling by centrifuging for 30 min at 120 × g and 4 °C. Na₂S₂O₅ and EDTA were added as preservatives in final concentrations of about 10 g/L each. Platelet concentrations were measured with a Coulter Counter Model S plus 4 (Coulter Electronics, Hialeah, FL). Later during the study, platelet-rich plasma was additionally separated into platelet-poor plasma and a pellet by centrifugation, as described by Crawford (16). Tissue samples were taken from biopsies obtained for necessary histopathological diagnostic procedures. One part was immediately transferred to a 0.01 mol/L acetic acid solution containing 10 g/L each of Na₂S₂O₅ and EDTA. Samples were stored at −20 °C and analyzed within one week after collection.

Analytical methods. The serotonin contents of platelet-rich plasma, platelet-poor plasma, urine, and tissue homogenates were determined by HPLC with fluoro-
metric detection, as described by Kwarts et al. (17). Platelet serotonin content, expressed in nanomoles of serotonin per 10⁹ platelets, was calculated by dividing the concentration of serotonin in platelet-rich plasma by the concentration of platelets in the plasma. Here we refer to the results of these calculations as “apparent platelet serotonin content.” Because the serotonin in platelet-rich plasma was not necessarily confined to platelets in all cases (see Discussion). Urinary 5-HIAA concentrations were determined in ether extracts by HPLC with fluorometric detection, essentially as described by Rosano et al. (18). Urinary creatinine concentrations were measured by a picric acid method with an SMA-2 analyzer (Technicon Instruments, Tarrytown, NY), and urinary 5-HIAA concentrations were expressed in mmol/mol creatinine.

Statistics and calculations. We calculated 95% confidence intervals by nonparametric analyses (19). To estimate the 5-HIAA production derived from a total body platelet pool of 4.4 and 20 nmol of serotonin per 10⁹ platelets, we applied a two-compartment system approximation (20), based on the following data: total body platelet pool, 1.6 × 10¹⁰ platelets (peripheral blood about 70%; splenic pool about 30% (21)), half-life of serotonin in platelets, 4.2 days (22–24).

Results

In the group of 26 healthy adults, the distributions of urinary 5-HIAA and serotonin excretion amounts and plasma serotonin concentration appeared to be skewed upward (nongaussian), whereas apparent platelet serotonin was skewed toward lower values. Reference values were therefore expressed as medians and 95% nonparametric confidence intervals. For healthy adults these were urinary 5-HIAA, 1.6 and 0.8–3.8 mmol/mol creatinine; urinary serotonin, 36 and 25–66 μmol/mol creatinine; apparent platelet serotonin content, 4.4 and 2.9–5.4 nmol/10⁹ platelets; and plasma serotonin concentration, not detectable (<0.5 nmol/L) and <0.5–33.3 nmol/L plasma.

Table 1 lists the pretherapeutic amounts of 5-HIAA and serotonin in various compartments, the site of the primary tumor, the location of any metastases, and associated carcinoid syndrome symptoms. In the 11 patients with foregut carcinoids, five had borderline to moderately increased apparent platelet serotonin contents, but only one had above-normal excretion of urinary 5-HIAA. All 14 patients with midgut carcinoid tumors showed markedly increased apparent platelet serotonin contents; in 13, urinary 5-HIAA excretions were grossly increased. In the five patients with hindgut carcinoid, one patient showed increased apparent platelet serotonin content; none had above-normal urinary 5-HIAA excretion. The urinary excretion of serotonin was above the upper limit of the confidence interval in 2 of 7, 10 of 13, and 2 of 5 patients with tumors of fore-, mid-, and hindgut origin, respectively.

Figure 1 depicts the relation between pretherapeutic urinary 5-HIAA excretion amount and apparent platelet serotonin content for all 30 patients with carcinoid tumors. In 10 we found no signs of increased serotonin production. Both urinary 5-HIAA and apparent platelet serotonin content were increased in 14 patients. Apparent platelet serotonin content but not the urinary 5-HIAA was increased in 6. None of the patients showed an increase in urinary 5-HIAA excretion amounts without increased apparent platelet serotonin content.

The serotonin contents of seven primary tumor biopsies and four metastases (Table 1) ranged from 0.011 to 12 500 and 0.006 to 36 400 nmol/g wet tissue, respectively. Primary foregut (n = 4; all lung; 0.011–98.83 nmol/g wet tissue) and hindgut (n = 1; rectum; 37.5 nmol/g wet tissue) carcinoids had lower serotonin contents than midgut carcinoids (n = 2; both ileal; 370 and 12 500 nmol/g wet tissue). In patients with midgut carcinoids this coincided with higher apparent platelet serotonin content, higher urinary 5-HIAA and serotonin values, higher serotonin content of metastases (n = 2; both liver; 28 400–36 400 nmol/g wet tissue), and the occurrence of carcinoid syndrome symptoms.

Figure 2 shows long-term monitoring of apparent platelet serotonin contents and urinary 5-HIAA excretion amounts for patients 14 and 21, both having liver involvement by a carcinoid of midgut origin, and for patient 4, who had a primary foregut (epiglottic) carcinoid and developing skin metastases (see Table 1). The initial clinical chemical diagnosis of patient 14 was based on increased apparent platelet serotonin content and increased urinary 5-HIAA and serotonin excretion amounts. This patient underwent resection of the primary tumor and a large liver metastasis in the left lobe. A small metastasis was left in situ in the right lobe. Postoperatively, urinary 5-HIAA excretion decreased dramatically, reaching transient stabilization at borderline values. Apparent platelet serotonin content also was reduced markedly after the operation but remained above the upper limit of the reference range during the entire postoperative period.
Patient 21 was initially biochemically characterized by increased apparent platelet serotonin content and normal concentrations of urinary 5-HIAA and serotonin. After resection of the primary tumor and lymph node metastases, the apparent platelet serotonin content decreased. During treatment with α-interferon, the apparent platelet serotonin content decreased further, whereas urinary 5-HIAA excretion remained within the reference range. After surgical removal of the only detectable liver metastasis, the apparent platelet serotonin content initially returned to normal, but subsequently steadily increased to just above normal values. Urinary 5-HIAA concentrations were virtually unaffected by tumor resection. Patient 21 has given no postoperative histological indication of tumor recurrence.

Patient 4 initially had a marginally increased platelet serotonin content and normal values for urinary 5-HIAA and serotonin excretion. During the course of his disease, multiple skin metastases appeared. This had no effect on urinary 5-HIAA excretion values, but platelet serotonin increased by >300%. Chemotherapy, although having no significant effect on the clinical status, caused a decrease in platelet serotonin content.

From the 27 patients studied longitudinally, we analyzed an average of 18 samples (range 2–47) for platelet serotonin, 13 samples (range 2–74) for urinary 5-HIAA, and 9 samples (range 1–29) for urinary serotonin. Apart from the effects of surgical or systemic treatment and strongly progressive disease, we observed no major changes in the ratio between platelet serotonin concentration and urinary 5-HIAA excretion, thus confirming the previous finding that platelet serotonin provided greater pretherapeutic sensitivity than did urinary 5-HIAA (data not shown).

Figure 3 shows the relation between apparent platelet serotonin content and the corresponding serotonin concentration in platelet-poor plasma for 26 healthy persons and 19 patients during different stages of various treatments. The data are derived from one sample from each healthy person and a mean of six samples (range: 1–14) per carcinoid patient. The results show that an increasing apparent platelet serotonin content in these patients was accompanied by increasing concentrations (>33.3 nmol/L) of serotonin in their platelet-poor plasma.

Discussion

Table 1 shows that, for 30 consecutive patients with carcinoid tumors referred to our hospital, apparent platelet serotonin content is a more sensitive marker for the diagnosis of carcinoid tumors of various origins than is the urinary 5-HIAA excretion value. This was especially true for patients with foregut carcinoids, who secrete only small amounts of serotonin. Thus, for the diagnosis of these patients, who often present without the symptoms of the carcinoid syndrome, measurement of platelet serotonin can be a valuable diagnostic aid. The courses of apparent platelet serotonin content and urinary 5-HIAA values in one of the longitudinally monitored patients (Figure 2A) suggest that increasing
serotonin production by a proliferating carcinoid tumor becomes at first noticeable from the apparent platelet serotonin content, whereas the urinary 5-HIAA excretion value exceeds the upper limit of the reference range at a later stage. This was also noticeable in three other patients, for whom longitudinal data were not shown.

Direct measurement of serotonin in platelet-rich plasma by HPLC with fluorometric detection, plus expression of results per number of platelets, yielded a reference range of 2.9–5.4 nmol/10⁹ platelets, which compares favorably with data from others using similar methods (25, 26). Although the median age of the control subjects (30 years) differs from that of the carcinoid patient group (59.5 years), it is reasonable to assume that the upper values of the analyses established for the control group are valid for the evaluation of the patient group. Platelet serotonin content is known to be age dependent, being about 30% lower in elderly subjects than in adults and children (27). For urinary 5-HIAA excretion no age dependency has been reported, whereas urinary creatinine excretion decreases with age (28). We therefore conclude that the difference in age between the control group and the carcinoid group has not biased our results.

Measurements of serotonin in urine, plasma, and platelets can give additional information for the diagnosis of patients with carcinoid tumors (12, 29). On the basis of one case with hyperserotonemia and normal urinary 5-HIAA excretion, Davis and Rosenberg (29) suggested that, in certain cases of carcinoid with small production of serotonin, measurement of total circulating serotonin may be of more diagnostic value than determination of urinary 5-HIAA excretion. In Feldman's study (12), 3 of 75 patients (2 with midgut carcinoid, 1 with carcinoid of unknown origin) had increased platelet serotonin contents in conjunction with normal values for urinary 5-HIAA and serotonin excretion; however, 11% of the patients had a combination of normal platelet serotonin content and increased urinary 5-HIAA excretion values. Perhaps the biochemical diversity of carcinoid tumors accounts for the discrepancy in sensitivity between the biochemical markers for carcinoid established in different studies (12, 14, 15).

The secretion rate of serotonin by a carcinoid tumor can obviously exceed the maximum uptake capacity of the total body platelet pool. As Figure 1 shows, despite increases in urinary 5-HIAA excretion to amounts >100-fold the upper limit of the reference range, apparent platelet serotonin content reaches a maximum at about 45 nmol/10⁹ platelets. In the body the surplus of serotonin secretion that is not taken up by platelets circulates freely until it is captured in endothelial cells of blood vessels (especially those of the lung), or in liver cells, where it is subsequently metabolized to 5-HIAA (30). Even after passage through lungs and liver—which, depending on their degree of adaptation to high concentrations of plasma serotonin, can remove up to 90% of all free circulating serotonin in a single passage (30)—plasma serotonin concentrations can be substantial (Figure 3). These data show that an increasing apparent platelet serotonin content measured in blood sampled from the cubital vein is accompanied by an increase in the concentration of serotonin in platelet-poor plasma. Crawford (16), who studied in vivo and in vitro uptake of serotonin by normal and "carcinoid" platelets, found that, above ~20 nmol/10⁹ platelets, platelet and plasma serotonin contents exhibit a linear relationship. At greater values he suggested that the platelet serotonin uptake mechanism involves passive diffusion, as opposed to an active serotonin uptake mechanism at normal serotonin secretion rates. Thus, this value probably reflects a serotonin secretion rate that, on the average, equals the capacity of the whole body platelet pool to accumulate the released serotonin without giving rise to significant plasma serotonin values. Thus, for patients with serotonin-producing carcinoids who have apparent platelet serotonin values of at least 20 nmol/10⁹ platelets, it is incorrect to ascribe all serotonin in platelet-rich plasma to the platelets for calculating the real platelet serotonin content. We therefore refer to this measurement as the "apparent platelet serotonin content." With increasing rates of serotonin secretion, apparent platelet serotonin contents finally plateau at about 45 nmol/10⁹ platelets. At this value, apparent platelet serotonin content does not relate to the serotonin secretion rate at all, whereas urinary 5-HIAA excretion still does.

Theoretically, platelet serotonin content could conceivably give more relevant information for the diagnosis of carcinoid tumors that secrete small amounts of serotonin than does the urinary 5-HIAA excretion value. Using a two-compartment system, we calculated that under normal conditions platelet turnover in the platelet serotonin pool produces 1.2 μmol of 5-HIAA daily. The total daily urinary excretion of 5-HIAA, which derives mainly from serotonin turnover in enterochromaffin cells of the gastrointestinal tract (31), is ~16.5 μmol. Assume that, per unit of time, a carcinoid tumor releases the amount of serotonin that can— together with serotonin from normal enterochromaffin tissue—be taken up by the total body platelet pool without giving rise to a significant amount of plasma serotonin. Further assume that the platelet serotonin content increases (as seen here) from 4.4 to 20 nmol/10⁹ platelets (i.e., by ~450%). As calculated with a two-compartment system, a platelet serotonin content of 20 nmol/10⁹ platelets would give rise to a daily production of 5.3 μmol of 5-HIAA, of which 4.1 μmol would originate from the carcinoid tumor. On top of the normal median daily urinary excretion of 16.5 μmol of 5-HIAA, this surplus corresponds to an increase in 5-HIAA of only 25%. Apart from the considerably higher increase in platelet serotonin content, this increase in urinary serotonin can be established with greater accuracy than platelet serotonin. The reference range for platelet serotonin content is relatively small and is not influenced by diet (12; and Kema et al., ms. accepted for publica-
tion in Clinical Chemistry), whereas the reference range of urinary 5-HIAA is wider. The latter is at least partly caused by dietary influences, e.g., ingestion of serotonin-rich foods such as bananas, pineapples, and walnuts (32). Serotonin catabolism (within the tumor or by other organs) before reaching platelets for uptake will decrease the above-calculated sensitivity of platelet serotonin and increase that of urinary 5-HIAA. In the present group of patients, however, we did not encounter cases that could be diagnosed on the basis of increased urinary 5-HIAA excretion only.

All but 2 of 14 patients exhibiting carcinoid syndrome (Table 1) had apparent platelet serotonin contents >20 nmol/10^9 platelets, which suggests that, when the tumor serotonin secretion rate exceeds the platelet uptake capacity, the resulting accumulation of serotonin in plasma can lead to some of the symptoms of the carcinoid syndrome. Serotonin overproduction has especially been implicated in the etiology of diarrhea and, in long-standing disease, valvular heart disease (1). The role of serotonin in flushing is disputed, because other mediators may also be involved (5, 33, 34). In our patient group, 12 of 14 patients with serotonin production rates beyond the platelet uptake capacity exhibited flushing.

Carcinoid tumors arising from the foregut may possess low activities of aromatic L-amino acid decarboxylase (35, 36), which converts 5-HTP into serotonin. Circulating 5-HTP is rapidly taken up by various organs that convert it to serotonin. The kidney is especially active in this process and subsequently excretes serotonin in urine (12, 37, 38). For the diagnosis of these cases, measurements of urinary excretion amounts of 5-HTP and serotonin have been proposed (39, 12). Platelets do not possess significant aromatic L-amino acid decarboxylase activity (40). However, the conversion of 5-HTP to serotonin by other tissues may contribute to the platelet serotonin pool, as suggested by experiments in which labeled 5-HTP was infused and labeled serotonin was recovered from platelets (22). From the 30 patients in our group, 14 exhibited increased urinary serotonin values (Table 1) that may predominantly have originated from circulating 5-HTP. All but one (no. 27) had increased apparent platelet serotonin content, whereas three exhibited normal urinary 5-HIAA excretion. We therefore conclude that, in general, platelet serotonin content is as informative as measurement of circulating or urinary 5-HTP. The low sensitivity of urinary serotonin in patients with foregut carcinoids does not support consistent excretion of 5-HTP by these tumors.

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