5-Hydroxyindoleacetic acid excretion following combination chemotherapy with cyclophosphamide, epirubicin and 5-fluorouracil plus ondansetron compared to ondansetron alone

Abstract The aim of the work was to evaluate the impact of cyclophosphamide and ondansetron on serotonin metabolism measured by urinary 5-hydroxyindoleacetic acid (5-HIAA) excretion. The pattern of urinary 5-HIAA excretion was analysed within 24 h following cyclophosphamide, epirubicin and 5-fluorouracil (FEC) chemotherapy \( (n = 14) \), ondansetron as single agent \( (n = 31) \), and in a control group \( (n = 62) \). 5-HIAA was measured by a fluorescence/polarisation immunoassay. Both FEC and ondansetron alone induced a significantly higher 5-HIAA increase following the first 12 h after drug administration when compared to the control group. The comparison of quantitative variables of 5-HIAA excretion between FEC and ondansetron failed to reveal any statistical differences. Cyclophosphamide-based chemotherapy is associated with only minor increases of 5-HIAA excretion. Analysis of 5-HIAA excretion does not help in the description of the pathophysiology of cyclophosphamide-induced emesis. In contrast to experimental data, serotonin 3 receptor antagonism with ondansetron induces an increase of 5-HIAA excretion in humans.

Key words Vomiting • Ondansetron • Cyclophosphamide • 5-Hydroxyindoleacetic acid (5-HIAA) • 5-HT3 antagonists

Introduction Cyclophosphamide-based chemotherapy combinations are frequently used for the treatment of solid tumours. Emesis is among the most distressing side-effects of cyclophosphamide-based chemotherapy and, without effective antiemetic prophylaxis, the vast majority of patients experience vomiting and nausea. The addition of anthracyclines enhances the emetogenic potential of cyclophosphamide. Emesis induced by combinations of cyclophosphamide and anthracycline is observed in 87%-97% of patients who have not received antiemetic prophylaxis [1, 9, 16]. Serotonin-3-receptor (5-HT3) antagonists have proven efficacy in the prophylaxis of cyclophosphamide-induced emesis in both clinical [1, 2, 5, 9, 15] and experimental settings [18]. The latter findings suggest that serotonin (5-HT) and 5-HT3 receptors are involved in the pathophysiology of cyclophosphamide-induced emesis. Analysis of the urinary excretion patterns of 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin, have shown marked increases of 5-HIAA excretion following the administration of cisplatin [7, 12, 20], carboplatin [13], dacarbazine [8] and nitrogen mustard [10]. Cyclophosphamide-based chemotherapy has been reported to be associated with only minor changes in 5-HIAA excretion in one study [8] whilst a recently published analysis from the same group failed to show any significant increase in 5-HIAA excretion following cyclophosphamide administration [10].

The administration of different antiemetics including 5-HT3 antagonists does not influence the pattern of
5-HIAA excretion following cisplatin chemotherapy [6, 11, 12, 20]. Comparable analysis in cyclophosphamide-treated patients is lacking and the influence of 5-HT₃ antagonists themselves on 5-HIAA excretion has not been analysed previously. Experimental data suggest a critical role for 5-HT₃ located on enterochromaffin cells, the main storage of total body 5-HT, in the control of 5-HT release. 5-HT₃ receptors showed an enhancing effect on 5-HT release from enterochromaffin cells in animal models [14]. Further experimental data suggest that 5-HT₃ antagonists might inhibit cisplatin-induced 5-HT release from enterochromaffin cells [19] as well as 5-HT release induced by 5-HT₃ agonists [17]. The tremendous increase of urinary 5-HIAA following cisplatin therapy might conceal the effect of 5-HT₃ receptor blockade on enterochromaffin cells in humans. Theoretically the effect of 5-HT₃ blockade could be important in chemotherapies which induce only minor changes in 5-HT metabolism, like cyclophosphamide-based regimens.

This study evaluates the impact of cyclophosphamid-based chemotherapy on the excretion pattern of 5-HIAA. Furthermore, we analyse the patterns of 5-HIAA excretion induced by 5-HT₃ antagonists without concomitantly administered chemotherapy. The latter group is compared with the cyclophosphamid-treated patients to separate the effects of cyclophosphamide and 5-HT₃ antagonists on 5-HIAA excretion.

**Patients, materials and methods**

Fourteen female patients who were treated with cyclophosphamide, epirubicin, and 5-FU (FEC) gave informed consent and were included. The mean age was 61 years (range 27–73 years). All patients were treated for metastatic breast cancer and received single-day i.v. FEC consisting of 600 mg/m² 5-FU given as a bolus injection, a 60-mg/m² epirubicin bolus injection, and 600 mg/m² cyclophosphamide diluted in 250 ml normal saline and infused over 1 h. Chemotherapy was administered at 10 a.m. on day 1. Antiemesis consisted of 8 mg ondansetron diluted in 50 ml normal saline and given as short infusion 30 min prior to chemotherapy. If the cyclophosphamide dose exceeded 1000 mg, uroprotection was gained with 200 mg sodium 2-mercaptoethanesulphonate (Mesna) administered before and 4 h and 8 h following cyclophosphamide infusion. A group of 31 volunteers who did not receive chemotherapy treatment gave informed consent and were enrolled in the ondansetron group. These patients suffered from breast cancer or gynaecological cancers and were scheduled to receive chemotherapy within the next week. The mean age in the control group was 62 (40–82) years.

The observation period started on the afternoon on day ~1 and lasted for at least 24 h following chemotherapy or ondansetron administration. All patients were treated as inpatients at the Universitaets Frauenklinik Freiburg. Emesis in the FEC group was documented by study nurses.

Urinary 5-HIAA was measured in urine samples collected over the whole observation period. Collection was every 4 h on the day before day 1. On day 1, urine collection was every 2 h. Collection periods were extended to 4 h on the night of day 1 and intervals were 8 h on the day before chemotherapy. Urine catheters were inserted on the day of chemotherapy in 2 patients who were not able to collect urine reliably. The urine was stored in dark containers and the urine volume of each sample was documented. A 10-ml aliquot was taken at the end of each period and was frozen at −20°C until measurement. 5-HIAA was measured with a fluorescence/polarisation immunoassay [4]. In short, urine samples were acidified and an organic extraction was performed. The organic phase was incubated with antibody serum and fluorescence tracer (5-HIAA FPIA kit, Abbott Diagnostics). Polarized light (481–489 nm) stimulates the fluorescence tracer, which leads to an emission of green light (525–550 nm) after a short period of latency. This emission was measured and compared to the background light intensity. Every measurement was accompanied by two control series. Pre-study quality tests showed a 90%–95% recovery of standard 5-HIAA probes (Sigma Chemicals) and control series with redundant measurements of standards showed a variation coefficient of less than 8%. Urine creatinine was measured with a radiation-energy-absorption assay (Abbott diagnostics). Creatinine forms a red-coloured complex with picric acid, which absorbs light in proportion to the creatinine concentration. The light absorption was measured by the Abbott TDx analyser. Pre-study tests showed a 99.6% correlation to biochemical standard tests and a variation coefficient below 5%. The 5-HIAA values were divided by the urine creatinine value in order to correct for inter- and intra-individual variation of urine concentrations [5-HIAA(µg/ml)/creatinine (mg/dl)].

The 5-HIAA/creatinine ratios measured after chemotherapy administration were compared to baseline levels (Δ5-HIAA/creatinine ratios) and represent the percentage deviation from the baseline values. A baseline value for each patient was calculated as the mean 5-HIAA/creatinine ratio of the three samples collected before drug administration. Chemotherapy infusion started at 10 a.m. and the baseline values were usually obtained from the collections between from 8 p.m. and midnight on the day before chemotherapy, between midnight and 8 a.m., and between 8 a.m. and 10 a.m. Further variables analysed were the Δ peak, which represents the highest 5-HIAA/creatinine ratio within the first 12 h following chemotherapy for each patient and Δ5-HIAA₀₋₁₂, which represents the median increase of 5-HIAA excretion in the first 12 h following drug administration. The Wilcoxon test was used for statistical comparison and significance was assumed for P<0.05.

**Results**

Emesis was observed in 4/14 patients of the FEC group whilst none of the ondansetron group or the control group reported emesis within the 24-h observation period. Emesis commenced early within the first 12 h following chemotherapy in all 4 patients.

All patients of the FEC group and the ondansetron group were evaluable for the analysis of 5-HIAA excretion patterns over the whole 24-hour observation period. The control group showed a significant drop-out...