Method for Individualized Evaluation of Antiemetic Effect Induced by 5-HT<sub>3</sub> Receptor Antagonist

Hironori Nakamura, Haruko Yokoyama, Koichi Yoshimoto, Akihiro Nakajima, Kiyoshi Okuyama, Osamu Iwase, and Yasuhiro Yamada

Department of Clinical Evaluation of Drug Efficacy, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences; 1432–1 Horinouchi, Hachioji, Tokyo 192–0392, Japan; Department of Pharmacy, Tokyo Medical University Hachioji Medical Center; and Department of Hematology, Tokyo Medical University Hachioji Medical Center; 1163 Tate-machi, Hachioji, Tokyo 193–0998, Japan.

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5-HT<sub>3</sub> receptor antagonists are widely used for prevention of chemotherapy-induced nausea and vomiting, though their antiemetic effects vary among patients. We investigated a method for evaluation of antiemetic effects in individual patients. We used the 5-HT<sub>3</sub> receptor occupancy of serotonin for our evaluation, which was estimated based on the plasma concentration of granisetron and concentration of serotonin near the 5-HT<sub>3</sub> receptor in the small intestine, obtained by measuring the urinary concentrations of granisetron and 5-hydroxyindoleacetic acid (5-HIAA)/creatinine (Cre). The mean cumulative percent for urinary excretion of granisetron at 24 h after administration and coefficient of variation were 16.19±6.30% and 38.91%, respectively. The time course of urinary concentration of 5-HIAA/Cre also varied among the patients. The value for 5-HT<sub>3</sub> receptor occupancy of serotonin without granisetron was higher than that prior to administration (blank), thus most treated patients had the possibility of induced emesis. In contrast, that with granisetron was lower than the blank value, indicating that those treated patients would not develop emesis. Furthermore, the estimated 5-HT<sub>3</sub> receptor occupancy of serotonin in the small intestine and actual individual patient condition corresponded well, showing the validity of our method. Our results suggest that it is possible to evaluate individual antiemetic effects by estimating the 5-HT<sub>3</sub> receptor occupancy of serotonin in the small intestine based on plasma concentrations of granisetron and serotonin near the 5-HT<sub>3</sub> receptor in the small intestine using noninvasive urine samples. This method of individual evaluation is considered to be useful and effective.

Key words urinary 5-hydroxyindoleacetic acid; 5-HT<sub>3</sub> receptor occupancy; granisetron; 5-HT<sub>3</sub> receptor antagonist; antiemetic effect

Chemotherapy-induced nausea and vomiting can lead to discontinuation of treatment and decrease the quality of life of affected patients. One of the main mechanisms of these adverse effects induced by antineoplastic agents is activation by serotonin of 5-HT<sub>3</sub> receptors, which exist on vagus nerve afferent fibers in the small intestine mucous membrane. With antineoplastic drug administration, serotonin is released from enterochromaffin cells, which synthesize and secrete this neurotransmitter, and stimulates 5-HT<sub>3</sub> receptors on adjacent vagal afferent nerves. Depolarization of the vagal afferent nerves stimulates the vomiting center in the brainstem and eventually induces a vomiting reflex. Therefore, the concentration of serotonin near 5-HT<sub>3</sub> receptors in the small intestine may be an indicator of the degree of emetic action. Serotonin is metabolized to 5-hydroxyindoleacetic acid (5-HIAA), which is subsequently excreted in urine. Moreover, a relationship between the frequency of vomiting and cumulative urinary excretion of 5-HIAA after administration of cisplatin (50 mg/m<sup>2</sup>) has been reported. As a result, the urinary concentration of 5-HIAA may also be a helpful indicator of the degree of emetic action.

5-HT<sub>3</sub> receptor antagonists are widely used as antiemetic drugs in clinical situations, with granisetron one of the major agents used throughout the world. However, it was reported that the area under the blood concentration–time curve (AUC) of granisetron is 63.06±36.54 ng/h/mL (mean±S.D., n=6, 40 µg/kg) and the coefficient of variation (CV%) was found to be 57.9%, indicating that individual variations are large. In addition, the plasma concentration of granisetron at 5 h after administration was significantly different between responders and non-responders. Moreover, we previously reported that interindividual variations for eating scores were large in patients administered granisetron. Thus, clinical difficulties may be encountered because of the wide interindividual variations in plasma concentrations and variable antiemetic effects of granisetron among patients.

The purpose of the present study was to develop a methodology for individual evaluation of the antiemetic effect of a 5-HT<sub>3</sub> receptor antagonist, using noninvasive urine sampling. Since some symptoms in cancer patients are severe, we consider that blood sample collections are more difficult over a short period of time and sought to develop a method by which the antiemetic effects of 5-HT<sub>3</sub> receptor antagonists could be predicted by testing urine samples.

We investigated a method for individual evaluations of antiemetic effects by estimating the 5-HT<sub>3</sub> receptor occupancy of serotonin (Φ<sub>A</sub>). For this purpose, we determined the concentration of granisetron in plasma and that of serotonin near the 5-HT<sub>3</sub> receptor in the small intestine by measuring the urinary concentrations of granisetron and 5-HIAA/Cre.

MATERIALS AND METHODS

The study protocol is shown in Fig. 1. We measured the
urinary concentration of granisetron and estimated its plasma concentration after intravenous administration, and also determined the urinary concentration of 5-HIAA and estimated the concentration of serotonin near the 5-HT3 receptor in the small intestine. Thereafter, we estimated the 5-HT3 receptor occupancy of serotonin ($\Phi_s$) in the small intestine based on the plasma concentration of granisetron and concentration of serotonin near the 5-HT3 receptor in the small intestine. We speculated that emesis is induced when the estimated 5-HT3 receptor occupancy of serotonin in the small intestine is greater than that prior to administration (blank). Thus, the antiemetic effect of granisetron was evaluated based on the blank level of 5-HT3 receptor occupancy of serotonin. When that estimated level is lower than the blank level, then we consider that the dosage of granisetron is appropriate, while a level higher than the blank is considered to indicate reconsideration of the dosage of granisetron and whether it is an appropriate agent for antiemetic therapy in that patient. In addition, we evaluated the effectiveness of our method by comparing the estimated 5-HT3 receptor occupancy of serotonin with food intake, and occurrence of nausea and vomiting in each patient.

Patients

In this study, we only sought to examine the antiemetic effects of a 5-HT3 receptor antagonist. Accordingly, we selected patients who were administered a 5-HT3 receptor antagonist alone as an antiemetic drug and not other agents such as neurokinin (NK) antagonists. Eight patients hospitalized at Tokyo Medical University Hachioji Medical Center and receiving anti-cancer chemotherapy for treatment of lymphoma gave informed consent and were enrolled in this study, which was performed according to the regulations of Tokyo Medical University and after receiving approval for the protocol from the ethics committee of that institution. Patient characteristics are shown in Table 1. There were no significant difference among them in regard to kidney and hepatic functions.

Patients received either R-CHOP or R-THP-COP as a chemotherapy regimen. Granisetron (3 mg/body) was intravenously administered over a period of 30 min or less on day 3 to all patients regardless of regimen. The ASCO guidelines ranks cyclophosphamide (<1500mg), doxorubicin, and pirarubicin as moderate emetogenic drugs, and rituximab and vincristine as minimal emetogenic drugs. For the present analysis, we did not discriminate between the chemotherapy regimens, as the standard dosage of cyclophosphamide is nearly the same between R-CHOP (750mg/m²) and R-THP-COP (650mg/m²).

Urine samples were collected at 24 h before (blank), and 0–2, 2–4, 4–6, and 6–24 h after administration of granisetron. Urine volume was measured, then each sample was immediately frozen and stored at −80°C until analysis.

Determination of Urinary Concentrations

For measuring the urinary concentration of granisetron, 1 mL of each urine sample was mixed with 0.5 mL of 0.1 m phosphoric acid buffer (pH 12) and 3 mL of toluene. This mixture was centrifuged and the supernatant was evaporated, with the residue dissolved with the mobile phase. The HPLC conditions were as follows: column, Luna 5 µm CN 100A (4.6 × 250 mm, 5 µm, SHIMADZU, Japan); mobile phase, methanol–0.05 m sodium acetate (pH 6.0) (4 : 1); detector, 300 nm for excitation and 353 nm for emission; quantification limit, 5 ng/mL.

We used the method reported by Yoshitake et al. to measure the urinary concentration of 5-HIAA, with some modifications. The HPLC conditions were as follows: column, CAPCELL PAK C18 (4.6 × 150 mm, 3 µm, Shiseido, Japan); mobile phase, acetoniitile–15 mM sodium acetate buffer (pH 5.5) (34:66) containing 3 mM sodium 1-octanesulfonate; detector, 345 nm for excitation and 480 nm for emission; quantification limit, 0.05 µg/mL.

For measuring the urinary concentration of creatinine, 20 µL of each urine sample was mixed with 980 µL of water.

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± S.D.</th>
<th>(Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>70.5 ± 5.8</td>
<td>(59.7–75.0)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>7/1</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.7 ± 7.2</td>
<td>(152–169.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>54.4 ± 9.8</td>
<td>(40.6–67.3)</td>
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<tr>
<td>Body surface area (m²)</td>
<td>1.57 ± 0.17</td>
<td>(1.35–1.77)</td>
</tr>
<tr>
<td>Scr (mg/dL)</td>
<td>0.71 ± 0.24</td>
<td>(0.17–0.93)</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>25 ± 5</td>
<td>(18–32)</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>21 ± 8</td>
<td>(15–37)</td>
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</table>
Estimation of Plasma Concentration of Granisetron

Granisetron is eliminated by hepatic metabolism and its unchanged urinary excretion ratio was reported to be 16.42±6.93% (n=6, 40 μg/kg d.i.v., after 48h) while a significant correlation between unchanged urinary excretion ratio and the AUC of granisetron (r=0.9032, p<0.001) has also been shown. We estimated the plasma concentration of granisetron based on its unchanged urinary concentration, using the following procedure. To estimate the parameters α, Vr, k12, and β, the time courses of plasma concentration and urinary excretion after administration of granisetron obtained in a phase I clinical trial were simultaneously fitted to Eq. 1 and Eq. 2 using a nonlinear least-square method. The results of Eq. 1 and Eq. 2 show the time course of plasma concentration after administration in a 2-compartment pharmacokinetic model and administration in a 2-compartment pharmacokinetic model and time course of amount of urine excretion, respectively.

\[
C_p(t) = \frac{k_0 \cdot (k_{21} - \alpha) \cdot \exp(-\alpha \cdot T) - 1}{W' \cdot V'_1 \cdot \alpha \cdot (\alpha - \beta)} \cdot \exp(-\alpha \cdot t') + \frac{k_0 \cdot (\beta - k_{21}) \cdot \exp(-\beta \cdot T) - 1}{W' \cdot V'_1 \cdot (\alpha - \beta)} \cdot \exp(-\beta \cdot t')
\]

\[
X_u(t) = \frac{A'}{100} \cdot D \cdot 1000 \cdot [1 - \exp(-\beta \cdot t)]
\]

where α (h⁻¹), β (h⁻¹), Cₚ (ng/mL), V₁ (L/kg), k₁₀ (h⁻¹), k₁₂ (h⁻¹), k₂₁ (h⁻¹), t' (h, t=t'+T), and Xₚ (ng) represent the elimination rate constant of the distribution phase, elimination rate constant of the elimination phase, plasma concentration of granisetron, central distribution volume per weight, elimination rate constant of granisetron, rate constant to the peripheral compartment from the central compartment, rate constant to the central compartment from the peripheral compartment, time after finish of administration, and amount of cumulative urinary excretion of granisetron, respectively. The relationship among α, β, and k₁₀, k₁₂, and k₂₁ was expressed as α+β=k₁₀+k₁₂+k₂₁, α=β=k₁₀/k₁₂. Furthermore, the values for infusion rate (k₀) (ng/h), weight (W) (kg), total infusion time (T) (h), dosage of granisetron (D) (μg), and cumulative urinary excretion ratio of granisetron at 24h after administration (A) (%) were 47800000 ng/h, 59.75 kg, 0.5 h, 2390 μg, and 15.4%, respectively, which were obtained in the phase I clinical trial. We used the MLAB nonlinear least squares program (Civilized Software Inc.) for analysis.

To estimate β' in each patient, the time course of the amount of cumulative urinary excretion of granisetron was fitted to Eq. 3 using a nonlinear least squares method.

\[
X_u(t) = \frac{A'}{100} \cdot D \cdot 1000 \cdot [1 - \exp(-\beta' \cdot t)]
\]

where A' (%) represents the individual cumulative urinary excretion ratio at 24h after administration of granisetron.

The common parameters (α, Vr, and k₂₁), individual parameters (W', k₀', A') and β' were substituted in Eq. 4 to estimate the time course of plasma concentration of granisetron.

Estimation of Concentration of Serotonin Near the 5-HT₁ Receptor in Small Intestine

To estimate the concentration of serotonin near the 5-HT₁ receptor in the small intestine, the measured amount of urinary 5-HIAA per 1 mg of creatinine (urinary 5-HIAA/Cre) in each patient was substituted in Eq. 5. It has been reported that blood serotonin originates from gastrointestinal mucosa and the total content in the gastrointestinal tract matches that of urinary 5-HIAA.

\[
C_i(t) = \frac{C_u \cdot f_S \cdot C_{\phi} \cdot tWV}{C_u^0}
\]

where Cᵢ (nm) and Cᵢ (μg/mg Cre) represent the concentration of serotonin near the 5-HT₁ receptor in the small intestine and urinary 5-HIAA/Cre, respectively. The values used for the concentration of serotonin in the small intestine (Cᵢ) (nm) and ratio of concentration of free serotonin to all serotonin in the small intestine (f₀) before administration of drugs were 850 nm and 0.01, respectively. The urinary 5-HIAA/Cre (C₀) (μg/mg Cre) value was used for determining urinary 5-HIAA/Cre in the blank samples.

Calculation of 5-HT₁ Receptor Occupancy of Serotonin in Small Intestine

To estimate the 5-HT₁ receptor occupancy of serotonin in the small intestine without granisetron (Φᵢ), the concentration of serotonin near the 5-HT₁ receptor in the small intestine was substituted in Eq. 6. Moreover, in order to estimate the 5-HT₁ receptor occupancy of serotonin in the small intestine with granisetron (Φᵢ'), the plasma concentration of granisetron and concentration of serotonin near the 5-HT₁ receptor in the small intestine were substituted in Eq. 7 (equation for reversible and competitive inhibition).

\[
\Phi_i = \frac{C_i}{k_i + C_i} \times 100
\]

\[
\Phi_i' = \frac{C_i}{k_i' \left[1 + \frac{C_p^f}{k_i' \phi_d}\right] + C_i} \times 100
\]

where, Φᵢ (%) and Φᵢ (%) represent the 5-HT₁ receptor occupancy of serotonin in the small intestine without and with granisetron, respectively, and Cᵢ (μg/mg Cre) is the free plasma concentration of granisetron (nm). Cᵢ (μg/mg Cre) is the dissociation constant of the 5-HT₁ receptor of serotonin (kᵢ) (nm), dissociation constant of the 5-HT₁ receptor of granisetron (kᵢ) (nm), and ratio of free granisetron in plasma (f₀) have been shown to be 150 nm, 0.41 nm, and 0.33, respectively.

Condition of Food Intake, and Occurrence of Nausea and Vomiting

We evaluated food intake and occurrence of nausea and vomiting in the patients based on data recorded from beginning of administration of granisetron to the finish of urine collection. Our previously reported method was used to quantify the condition of food intake, with some modifications. Eating scores for consumption of all, half, 1/3, a little,
and none were recorded as 5, 4, 3, 2, and 1, respectively, while nausea scores for none, a little, sometimes, continuing, and continuing serious were 5, 4, 3, 2, and 1, respectively. Vomiting occurrence was evaluated based on frequency and time.

RESULTS

The time course of plasma concentration of granisetron after administration (40 µg/kg) and cumulative amount of its urinary excretion in the phase I clinical trial were simultaneously fitted to Eq. 1 and Eq. 2. The observed values and the fitted curve were well matched. The pharmacokinetic parameters of $\alpha$, $\beta$, $V_1$, and $k_{21}$ for granisetron were estimated to be $2.80 h^{-1}$, $0.21 h^{-1}$, $1.49 L$, and $1.33 h^{-1}$, respectively.

The cumulative amount of urinary excretion of granisetron at 24 h after administration and the fitted curve for each patient are shown in Fig. 2. The fitted curves were matched to the observed data. The average values for cumulative urinary excretion ratio of granisetron at 24 h after administration ($A'$) and CV% were $16.19\pm6.30\%$ ($9.22\text{--}25.82\%$) and $38.91\%$, respectively, while the estimated value for parameter $\beta'$ was $0.18\pm0.10 h^{-1}$ ($0.117\text{--}0.347 h^{-1}$).

The time courses of the estimated plasma concentrations of granisetron, which differed in each patient, are shown in Fig. 3. The average values for $C_{\text{max}}$ (0.5 h after administration) and $t_{1/2\beta}$ were $27.85\pm8.16 ng/mL$ ($15.20\text{--}39.21 ng/mL$) and $4.59\pm1.63 h$ ($2.00\text{--}6.17 h$), respectively.

The individual values for time curve of urinary 5-HIAA/Cre, which differed in each patient, are shown in Fig. 4. The value after administration of antineoplastic drugs was increased as compared to that before administration in all except for patient No. VIII.

The estimated 5-HT$_3$ receptor occupancies of serotonin in the small intestine without ($\Phi_s$) or with ($\Phi_{sG}$) granisetron are...
shown in Fig. 5. That without granisetron after administration of drugs was higher than that before administration in all except for patient No. VIII, whereas that with granisetron after administration was lower than prior to administration (gray area) in all patients.

The average eating and nausea scores were $4.8 \pm 0.3$ and $4.9 \pm 0.1$, respectively, which were considered to indicate a good condition in the patients with granisetron. We considered that it would be unethical to perform the present protocol without granisetron.

**DISCUSSION**

We investigated a method for individual evaluation of antiemetic effect based on the $5\text{-HT}_3$ receptor occupancy of serotonin, which was estimated using the plasma concentration of granisetron and concentration of serotonin near the $5\text{-HT}_3$ receptor in the small intestine. Those values were obtained by measuring the urinary concentrations of granisetron and 5-HIAA/Cre.

Prednisolone in the R-CHOP and R-THP-COP regimens is administered for its antineoplastic effect. Tanaka et al. reported that a combination of prednisolone (500mg/d) and granisetron (40µg/d) had a greater antiemetic effect than
granisetron alone. However, because the dosage of prednisolone (30–60 mg/m²) was quite low in the present study, we think that it had only a scant antiemetic effect.

We determined the urinary concentration of granisetron after intravenous administration and then estimated the time course of its concentration in plasma in individual patients using calculations based on parameter data obtained in a phase I clinical trial. The estimated value for $V_1$ was corrected by $W'$, while $A'$ was the measured value and $\beta$ the individual value calculated from the measured value. Thus, we consider that our method can be used for individual subjects.

The mean cumulative amount of urinary excretion of granisetron at 24 h after administration was 16.19±6.30%, which was equal to that obtained in the phase I clinical trial (15.41%, granisetron 40 µg/kg). Furthermore, the mean calculated $C_{\text{max}}$ value in our study was 27.85±8.16 ng/mL, while that in the phase I clinical trial was 19.48±6.05 ng/mL (granisetron 40 µg/kg, d.i.v.). Since the dose of granisetron differed, it was corrected. If the $C_{\text{max}}$ value for granisetron in the phase I clinical trial (40 µg/kg) was calculated proportionality using the dose (55 µg/kg) employed in our study, that value would be 26.79 ng/mL, which is very similar to the value in the present study. Moreover, there were no significant differences for the mean of $t_{1/2}$β between this study and the phase I clinical trial (4.59±1.63 vs. 3.14±1.20 h). Therefore, it is suggested that our method for estimating the individual plasma concentration of

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**Fig. 4. Urinary Concentration of 5-HIAA/Creatinine after Administration of Antineoplastic Drugs with Granisetron**
granisetron based on the amount of urinary excretion is appropriate. The CV% value for the amount of urinary excretion of granisetron varied widely and the time course of plasma concentration of granisetron had individual profiles for our patients. It is suggested that these differences account for the individual differences in antiemetic effects of granisetron and we consider that it is possible to evaluate individual differences of the pharmacokinetics of granisetron by estimating the time course of plasma concentration using our method.

We also measured the urinary concentration of 5-HIAA and estimated the concentration of serotonin near the 5-HT₃ receptor in the small intestine. As noted above, nearly all of released serotonin is immediately metabolized to 5-HIAA. Accordingly, we speculated that nearly all 5-HIAA excreted in urine is the result of metabolism of serotonin released from enterochromaffin cells. Furthermore, we considered that the urinary concentration of 5-HIAA reflects the concentration of serotonin near the 5-HT₃ receptor in the small intestine. Since quantitative determination of serotonin near the 5-HT₃ receptor in the small intestine in humans is impossible, we did not examine the relationship between estimated and actual values. Nevertheless, we consider that our evaluation was adequate, since it was used to compare antiemetic effects in comparison to the blank value for concentration of serotonin near the 5-HT₃ receptor in the small intestine.

The time course of the urinary concentration of 5-HIAA/Cre varied among the patients. The maximum 5-HIAA/Cre concentration time ($T_{\text{max}}$) was about 4h after administration.
of the antineoplastic drug in 4 patients, which is quite similar to another report that found that $T_{\text{max}}$ of 5-HIAA/Cr was reached about 4h after administration of cyclophosphamide (520±30 mg/m²) in cancer patients.\(^{16}\) On the other hand, there was no salient peak of urinary 5-HIAA/Cr concentration in some of the patients, which is consistent with a report in which no salient peak of urinary 5-HIAA/Cr concentration was found up to 24h after administration of cyclophosphamide (500 mg/m²) in cancer patients.\(^{15}\) Thus, the behavior of serotonin released after administration of cyclophosphamide is different in each report and there were also individual differences in our study.

In addition, we estimated the 5-HT\(_3\) receptor occupancy of serotonin in the small intestine using the plasma concentration of granisetron and concentration of serotonin near the 5-HT\(_3\) receptor in the small intestine. Our results showed that the 5-HT\(_3\) receptor occupancy of serotonin without granisetron was higher than the blank value and none of those patients except 1 were thought to have emesis. On the other hand, the 5-HT\(_3\) receptor occupancy of serotonin with granisetron was lower than the blank value and all of those patients had emesis. The mean eating and nausea scores indicated a good condition, and none of our patients experienced vomiting. Therefore, the estimated 5-HT\(_3\) receptor occupancy of serotonin in the small intestine and actual individual patient condition showed good correspondence, and the efficacy of granisetron was shown.

In conclusion, we developed a methodology for predicting the antiemetic effects of a 5-HT\(_3\) receptor antagonist based on urinary sample measurements. Our results also suggest that it is possible to evaluate individual antiemetic effects by estimating the 5-HT\(_3\) receptor occupancy of serotonin in the small intestine using the plasma concentration of granisetron and concentration near the 5-HT\(_3\) receptor in the small intestine using a noninvasive urine sample. Our method can also be useful to evaluate the antiemetic effects of 5-HT\(_3\) receptor antagonists in both quantitative and theoretical manners. Therefore, we consider that it could be utilized to determine an appropriate antiemetic therapy in clinical situations. Furthermore, it is also possible to predict antiemetic effects when considering changing to another 5-HT\(_3\) receptor antagonist. However, no vomiting patient was found in the present study. We intend to investigate a greater number of cancer patients as well as other chemotherapy regimens using this method in a future study.

REFERENCES