(s, C-11c), 128.25 (s, C-7a), 129.44 (s, C-3a), 131.68 (s, C-11a), 145.24 (s, C-2), 147.01 (s, C-9*), 147.13 (s, C-10*), and 152.60 ppm (s, C-1). Assignments are interchangeable between carbons with an asterisk. The MS and UV spectra were identical with published spectra (11, 13).

Bioassay—Male albino rabbits (2–3 kg) were killed by cervical dislocation. The aortae were removed and cut into helical strips. The strips were mounted vertically in a 20-mL organ bath containing Krebs–Ringer bicarbonate solution (pH 7.4) of the following composition (in mmoles): sodium chloride, 120; potassium chloride, 4.8; calcium chloride, 1.2; magnesium sulfate, 1.3; monobasic potassium phosphate, 1.2; sodium bicarbonate, 25.2; and glucose, 5.8. The solution was bubbled with a gas mixture of oxygen–carbon dioxide (95:5, v/v) and maintained at 37°C. A resting tension of 1 g was applied to the strips and isometric contractions were recorded with a force displacement transducer.

Male Wistar rats (300–350 g) were killed by a blow on the neck and the stomach was removed. The fundus was cut longitudinally into strips (20 × 2 mm) as described by Offermeiser and Ariens (19). The strips were suspended in a 20-mL organ bath containing Krebs–Ringer bicarbonate solution. The solution was aerated with oxygen–carbon dioxide (95:5 v/v) and maintained at 37°C. A resting tension of 0.5 g was applied to the strips. Mechanical responses were recorded isotonically on a pen recorder through an isotonic transducer.

RESULTS AND DISCUSSION

A methanolic extract of the fruit of *N. domestica* almost completely inhibited the serotonin-induced contractions of isolated rabbit aorta and had no effect on contractions induced by potassium chloride or histamine. To isolate the active substance, fractionation of the methanolic extract of the fruits were performed (Scheme 1), accompanied by a bioassay using isolated rabbit aorta. Silica gel chromatography of the *n*-butyl alcohol-soluble portion of the methanolic extract afforded the active substance as colorless crystals (8.0 g, 0.2% dry weight of the fruit). The compound showed a positive Dragendorff test and its physicochemical properties (*i.e.*, melting point, specific optical rotation, UV absorption, and mass spectrum) agreed with those of nantenine (1, 5, 6, 11, 13), which was previously isolated as a major alkaloid of the same material. Furthermore, the ^{1}H- and ^{13}C-NMR spectra also supported the identity of the active substance as nantenine (see *Experimental*).

In isolated rabbit aorta, nantenine (3×10^{-6} M) produced a parallel, right shift of the dose–response curve for the contractile effect of serotonin, but had no effect on the dose–response curves for histamine and potassium chloride (Fig. 1), indicating competitive antagonism. Furthermore, in rat stomach strips, nantenine (3×10^{-6} M) shifted the dose–contractile response curve for serotonin to the right in a similar manner, but the dose–response curves for carbachol and potassium chloride were not affected by nantenine (Fig. 2). These results suggest that nantenine selectively inhibits the contractile response of these tissues to serotonin. On the basis of the present results, it is concluded that *N. domestica* Thunberg has a serotonergic receptor blocking action in the isolated rabbit aorta, and that the main active compound is nantenine.

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COMMUNICATIONS

Computation of Model-Independent Pharmacokinetic Parameters During Multiple Dosing

Keyphrases □ Pharmacokinetic parameters—model independent, multiple dosing

To the Editor:

In a recent article, Bauer and Gibaldi (1) reported an alternate, noncompartmental method to calculate pharmacokinetic parameters during multiple dosing. The method was based on reverse superposition from which a single-dose drug concentration–time curve was derived from data obtained at steady state. The following method would be a more general approach for computation of model-independent pharmacokinetic parameters during multiple dosing. The plasma drug concentration–time curve after the Nth dose (C_N) of a fixed dose of a drug at a given dosing interval of τ can be described by Eq. 1, when a drug obeys linear pharmacokinetics:

$$C_N = \sum_{i=1}^{n} A_i \frac{1 - \exp(-Nk_i\tau)}{1 - \exp(-k_i\tau)} \exp(-k_i t) = \sum_{i=1}^{n} I_i \exp(-k_i t) \quad \text{(Eq. 1)}$$

where A_i and I_i are the coefficients of the specific first-order rate constant, k_i, after a single dose and the Nth dose, respectively; t is the time after each drug administration. The total area under the plasma drug concentration–time curve (AUC) from the time after the Nth dose is given to time infinity, $\text{AUC}(\infty)_N$ can be obtained as follows:

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$$\mathrm{AUC}(\infty)_N = \int_0^{\infty} C_N dt = \sum_{i=1}^{n} \frac{I_i}{k_i} = \mathrm{AUC}(\infty) + \sum_{i=1}^{n} \frac{A_i[1 - \exp(-(N-1)k_i\tau)\exp(-k_i\tau)]}{k_i[1 - \exp(-k_i\tau)]} = \mathrm{AUC}(\infty)_{ss} - \sum_{i=1}^{n} \frac{A_i \exp(-Nk_i\tau)}{k_i[1 - \exp(k_i\tau)]} \quad \text{(Eq. 2)}$$

When the dosing interval is located in the terminal phase, Eq. 2 can be approximated by Eq. 2a:

$$\mathrm{AUC}(\infty)_N = \mathrm{AUC}(\infty) + C_{\min N-1}/k_n = \mathrm{AUC}(\infty)_{ss} - (C_{\min ss} - C_{\min N-1})/k_n \quad \text{(Eq. 2a)}$$

where $\mathrm{AUC}(\infty)$ and $\mathrm{AUC}(\infty)_{ss}$ are the total areas after a single dose and at steady state, respectively; $C_{\min N-1}$ and $C_{\min ss}$ are the plasma drug concentrations at the dosing interval after the $(N-1)$th dose and at steady state, respectively; k_n is the terminal phase disposition rate constant.

The $\mathrm{AUC}(\infty)_N$ can be obtained by numerical integration using the trapezoidal rule from the time course data of plasma drug concentrations:

$$\mathrm{AUC}(\infty)_n = \int_0^{t^*} C_N dt + \int_{t^*}^{\infty} C_N dt = \sum_{i=1}^{n} \frac{1}{2}(C_{Ni} + C_{Ni-1})(t_i - t_{i-1}) + \sum_{i=1}^{n} \frac{I_i \exp(-k_i t^*)}{k_i} \quad \text{(Eq. 3)}$$

The residual area from t^* (usually the last sampling time point) to infinity is usually approximated by C_N^*/k_n, where C_N^* is the estimated drug concentration at time t^* after the Nth dose.

The total area under the first moment of the plasma drug concentration–time curve after the Nth dose, $\mathrm{AUMC}(\infty)_N$ can be calculated as follows:

$$\mathrm{AUMC}(\infty)_N = \int_0^{\infty} tC_N dt = \sum_{i=1}^{n} \frac{I_i}{k_i^2} = \mathrm{AUMC}(\infty) + \sum_{i=1}^{n} \frac{A_i[1 - \exp(-(N-1)k_i\tau)\exp(-k_i\tau)]}{k_i^2[1 - \exp(-k_i\tau)]} = \mathrm{AUMC}(\infty)_{ss} - \sum_{i=1}^{n} \frac{A_i \exp(-Nk_i\tau)}{k_i^2[1 - \exp(-k_i\tau)]} \quad \text{(Eq. 4)}$$

When the τ is located in the terminal phase, Eq. 4 can be approximated by Eq. 4a:

$$\mathrm{AUMC}(\infty)_N = \mathrm{AUMC}(\infty) + C_{\min N-1}/k_n^2 = \mathrm{AUMC}(\infty)_{ss} - (C_{\min ss} - C_{\min N-1})/k_n^2 \quad \text{(Eq. 4a)}$$

where $\mathrm{AUMC}(\infty)$ and $\mathrm{AUMC}(\infty)_{ss}$ are the total area moments after a single dose and at steady state, respectively. The $\mathrm{AUMC}(\infty)_N$ can also be obtained as follows:

$$\mathrm{AUMC}(\infty)_N = \sum_{i=1}^{n} \frac{1}{2}(t_i C_{Ni} + t_{i-1}C_{Ni-1})(t_i - t_{i-1}) + \sum_{i=1}^{n} \frac{I_i}{k_i^2}(1 + k_i t^*)\exp(-k_i t^*) \quad \text{(Eq. 5)}$$

The residual area moment from t^* to infinity is usually approximated by:

$$\frac{C_N^*}{k_n^2} + \frac{t^* C_N^*}{k_n}$$

According to the statistical concept of moments for pharmacokinetics, the mean residence time of a drug in the body (MRT) after a single dose is defined as follows (2):

$$\mathrm{MRT} = \frac{\mathrm{AUMC}(\infty)}{\mathrm{AUC}(\infty)} = \frac{\sum_{i=1}^{n} A_i/k_i^2}{\sum_{i=1}^{n} A_i/k_i} \quad \text{(Eq. 6)}$$

This concept can be extended to the observed data after the Nth dose and at steady state by applying Eqs. 2a and 4a as follows:

$$\mathrm{MRT} = \frac{k_n^2\,\mathrm{AUMC}(\infty)_N - C_{\min N-1}}{k_n[k_n\mathrm{AUC}(\infty)_N - C_{\min N-1}]} = \frac{k_n^2\mathrm{AUMC}(\infty)_{ss} - C_{\min ss}}{k_n[k_n\mathrm{AUC}(\infty)_{ss} - C_{\min ss}]} \quad \text{(Eq. 7)}$$

For the data obtained during the dosing interval:

$$\mathrm{MRT} = \frac{k_n^2\mathrm{AUMC}(\tau)_N + k_n\tau C_{minN} + C_{minN} - C_{minN-1}}{k_n[k_n\mathrm{AUC}(\tau)_N + C_{minN} - C_{minN-1}]} = \frac{k_n\mathrm{AUMC}(\tau)_{ss} + \tau C_{\min ss}}{k_n\mathrm{AUC}(\tau)_{ss}} = \frac{\mathrm{AUMC}(\tau)_{ss}}{\mathrm{AUC}(\tau)_{ss}} + \frac{\tau C_{\min ss}}{k_n\mathrm{AUC}(\tau)_{ss}} \quad \text{(Eq. 8)}$$

Although Bauer and Gibaldi (1) stated that the apparent volume of distribution at steady state, Vd_{ss}, cannot be calculated directly from steady-state data, Vd_{ss} can be calculated from the MRT (using Eq. 7 or Eq. 8) after multiple intravenous bolus dosing of a drug as (3):

$$Vd_{ss} = \mathrm{TBC}\cdot\mathrm{MRT} \quad \text{(Eq. 9)}$$

where total body clearance (TBC) after the Nth dose and at steady state can be calculated as:

$$\mathrm{TBC} = \frac{k_n\cdot\mathrm{Dose}}{k_n\mathrm{AUC}(\infty)_N - C_{\min N-1}} = \frac{\mathrm{Dose}}{\mathrm{AUC}(\tau)_{ss}} \quad \text{(Eq. 10)}$$

No model has to be assumed to calculate MRT, Vd_{ss}, and TBC after multiple dosing. This method can be applied to data obtained from any number of doses including steady state and does not require the plasma drug concentrations derived by means of reverse superposition. The method describes an exact solution and its approximation. The approximation requires an assumption that doses must be administered during the terminal phase. This assumption is generally valid. When the assumption is not valid, one may use the exponential equations to calculate the residual area and area moment.

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