Analysis of Pharmacological Effects of Drugs Used for Treatment of Urinary Disturbance Based on Anticholinergic and Smooth Muscle-Relaxing Effects

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Propiverine hydrochloride, oxybutynin hydrochloride and terodiline hydrochloride have both anticholinergic and antispasmodic effects, and are used for the management of urinary frequency and incontinence. The average standard therapeutic doses of these drugs differ greatly. We retrospectively analyzed their pharmacological effects with consideration given to muscarinic acetylcholine receptor binding affinities, anticholinergic activities, and inhibitory effects on KCl-induced contraction. Muscarinic acetylcholine receptor occupancies and the inhibitory ratios of the drugs for both acetylcholine-induced and KCl-induced contraction in a steady state after oral administration of standard doses were calculated based on pharmacokinetics and the receptor occupancy theory. The average muscarinic acetylcholine receptor occupancy and inhibitory ratio of acetylcholine-induced contraction were estimated to be 12.6±1.06% and 3.27±0.74%, respectively, with no significant differences found between the drugs for those parameters. A significant linear relationship was found between muscarinic acetylcholine receptor occupancy and the maximum ratio of increase in bladder urinary capacity. On the other hand, the inhibitory ratios of KCl-induced contraction varied from 0.01 to 0.48%. The present results suggest that muscarinic acetylcholine receptor occupancy is a principal determinant of the therapeutic effect of a drug used for treatment of urinary disturbance.

Key words muscarinic acetylcholine receptor; receptor occupancy; bladder urinary capacity; propiverine; oxybutynin; terodiline

Drugs for the treatment of urinary disturbance with anticholinergic and smooth muscle-relaxing effects are used for the management of urinary frequency and incontinence. Oxybutynin hydrochloride, propiverine hydrochloride, terodiline hydrochloride, and flavoxate hydrochloride have been marketed in Japan as drugs for treatment of urinary disturbance, and tolterodine tartrate and solifenacine succinate were put on the market in 2006. Sales of terodiline hydrochloride, however, have been stopped because of its adverse arrhythmic effect. The average standard therapeutic doses of the remaining available drugs differ greatly. In regard to anticholinergic and smooth muscle-relaxing effects of oxybutynin, propiverine, and tolterodine, it has been reported in studies using extracted rabbit bladders that in order to nearly completely inhibit contraction of the musculus detrusor urinae, each of those effects independently was insufficient and both were required.1,2) Those results were from in vitro pharmacological experiments, while there is no known report that considered the inhibitory actions toward musculus detrusor urinae contraction using oral administration of the drugs with standard doses.

We investigated the anticholinergic and antispasmodic effects of oxybutynin hydrochloride, propiverine hydrochloride, and terodiline hydrochloride to inhibit bladder smooth muscle contraction, by calculating the muscarinic acetylcholine receptor occupancies and inhibitory ratios of both acetylcholine-induced and KCl-induced contractions in a steady state after oral administrations of standard doses.

MATERIALS AND METHODS

Pharmacokinetic and Pharmacodynamic Parameters

Values for the primary pharmacokinetic parameters, i.e., total body clearance (CLtot), bioavailability (F), area under the plasma concentration time curve (AUC0–∞), and plasma unbound fraction (fu), were obtained from previous reports.3–8) The receptor dissociation constant (Kd) and anticholinergic activity (Ki) values were also obtained as data for the muscarinic acetylcholine receptor binding affinity of the drugs from literature.9–15) Ki values were determined by in vitro radiolabeled ligand binding inhibition experiments using guinea pig bladders, while Kd values were determined by in vitro pharmacological experiments with guinea pig bladders. The inhibitory effects of KCl-induced contraction (IC50) values were also obtained as data regarding the direct effects of the drugs on smooth muscle from a previous study.16) IC50 values were determined by in vitro pharmacological experiments using rat bladders.

Analysis of Muscarinic Acetylcholine Receptor Occupancy and Inhibitory Ratio of Acetylcholine-Induced Contraction

The muscarinic acetylcholine receptor occupancy and inhibitory ratio of acetylcholine-induced contraction of each drug for urinary disturbance following oral administration with a standard dose were calculated using data obtained as noted above. We assumed that the drugs used for urinary disturbance bound competitively to muscarinic acetylcholine receptors and expressed the concentration (nm) of bound receptors (R) using the following equation:

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R = \frac{R_{max} \cdot C_U}{C_U + Kd_d(1 + C_M/Kd_d)} + \frac{R_{max} \cdot C_M}{C_M + Kd_d(1 + C_U/Kd_d)}
\]

where \(R_{max}\) is the maximum concentration (nm) at the receptors; \(C_U\) and \(C_M\) are the concentrations (nm) of the unchanged drug and its metabolite, respectively, at the juxta-receptors;
and $K_d$ and $K_dM$ are the receptor dissociation constants, corresponding to $K_p$ or $K_u$ of the drug. We assumed that the substances acted as full antagonists toward muscarinic acetylcholine receptors and expressed the receptor occupancy, $\Phi(\%)$, according to the receptor occupation theory using the following equation:

$$\Phi = \frac{R}{R_{\text{max}}} \times 100$$

or

$$\Phi = \left( \frac{C_U}{C_U + K_dU(1 + C_M / K_dM)} \right) + \left( \frac{C_M}{C_M + K_dM(1 + C_U / K_dU)} \right) \times 100$$ (2)

After the drug has been absorbed into the bloodstream from the gastrointestinal tract, it passes across blood capillary cell walls into extracellular space and binds to muscarinic acetylcholine receptors on the plasma membranes of bladder cells. If the process of transport of the drug to endothelial cells is not active, but rather passive diffusion, or it is not a rate-limiting step because of fenestration on the blood capillary cell walls, we assumed that the concentration of the drug in the extracellular space in the bladder would not be significantly different from the unbound drug concentrations in plasma. Therefore, we used the unbound drug concentrations ($C_U^f$ and $C_M^f$) as the concentrations for the receptor-binding drugs ($C_U$ and $C_M$), respectively. The steady-state drug concentration in plasma was calculated from $CL_{\text{tot}} / F$ or $AUC_{\text{tot}} / \tau$, as follows:

$$C_{u}^f = f_u \cdot \frac{Dose \cdot F}{CL_{\text{tot}} \cdot \tau} = f_u \cdot \frac{AUC_{\text{tot}}}{\tau}$$ (3)

where $\tau$ represents the dosing interval. In order to estimate the receptor occupancy of the drug in a steady state, we calculated $C_{u}^f$ from $CL_{\text{tot}} / F$ or $AUC_{\text{tot}} / \tau$ using Eq. 3. We determined the receptor occupancy of each drug by using Eq. 2. Then, we examined the relationships of receptor occupancy and inhibitory ratio using the pharmacodynamic parameters.

It is known that oxybutynin and propiverine each have one pharmacologically active metabolite. However, there are no available data on the metabolites of oxybutynin, thus only data on the unchanged drug were used for the analysis of oxybutynin. Terodiline has no pharmacologically active metabolites.

Analysis of Inhibitory Ratio of KCl-Induced Contraction The inhibitory ratio of KCl-induced contraction for each of the drugs following oral administration with the standard dose was calculated using Eqs. 1—3. $K_d$ was calculated from the $IC_{50}$ value. The relationships between inhibitory ratio and pharmacodynamic parameters were then examined.

### Analysis of Relationships of Receptor Occupancy and Inhibitory Ratio of Contraction with Clinical Effect

The muscarinic acetylcholine receptor occupancy and inhibitory ratio of contraction of each of the drugs following oral administration of a dose found acceptable in clinical trials were calculated using Eqs. 1—3. We then examined the relationships of receptor occupancy and inhibitory ratio with clinical effect, which was defined as the maximum ratio of increase in bladder urinary capacity, data for which were obtained from clinical trial reports.

### Pharmacokinetic and Pharmacodynamic Parameters

Table 1 shows the values of $AUC_{\text{tot}}$, $f_u$, and $C_{u}^f$ following oral administration with standard doses, as well as the values of $K_1$ and $K_2$ for the anticholinergic effects, and $IC_{50}$ for the muscle-relaxing effects. The values of $IC_{50}$ did not vary greatly, whereas the differences between the minimum and maximum values of both $K_1$ and $K_2$ were nearly 100-fold.

### Receptor Occupancy and Inhibitory Ratio of Contraction in a Steady-State Condition

Receptor occupancies of propiverine, oxybutynin, and terodiline were 12.9%, 11.4%, and 13.5%. Inhibitory ratios of acetylcholine-induced contraction were 3.29%, 2.52%, and 3.99%, and inhibitory ratios of KCl-induced contraction were 0.25%, 0.01%, and 0.48%. Figure 1 shows the relationships of the pharmacodynamic parameters with muscarinic acetylcholine receptor occupancies, and inhibitory ratios for both acetylcholine-induced and KCl-induced contractions in a steady state after oral administration of standard doses of the three drugs. The average muscarinic acetylcholine receptor occupancy and average inhibitory ratio of acetylcholine-induced contraction were estimated to be 12.6±1.06% and 3.27±0.74%, respectively, while the inhibitory ratios of KCl-induced contraction varied from 0.01 to 0.48%.

### Relationships of Receptor Occupancy and Inhibitory Ratio of Contraction with Clinical Effect

Figure 2 shows the relationships of receptor occupancy and inhibitory ratio of contraction with the maximum ratio of increase in bladder urinary capacity. The relationship between muscarinic acetylcholine receptor occupancy and maximum ratio of increase in bladder urinary capacity was statistically significant ($p<0.05$). A correlation ($p<0.1$) was also found between acetylcholine-induced contraction and maximum ratio of increase in bladder urinary capacity. However, no significant relationship was found between the inhibitory ratio of
KCl-induced contraction and maximum ratio of increase in bladder urinary capacity.

DISCUSSION

In the present study, we investigated the contributions of anticholinergic and antispasmodic effects of three drugs used for management of urinary frequency and incontinence in regard to their inhibitory actions toward bladder smooth muscle contraction. Although these drugs were put on the market about 15—20 years ago, the quantity and quality of information for each drug was different.

It is more convenient to use values for $K_I$, $K_B$, and IC$_{50}$ obtained from experiments with guinea pigs and IC$_{50}$ values from those with rats as data for muscarinic acetylcholine receptor binding affinity and the inhibitory ratio of KCl-induced contraction, respectively. The relationships between $K_I$ values obtained from guinea pigs and humans, and between $K_I$ values obtained from rabbits and humans are shown in Fig. 3. Nevertheless, additional assessments of the correlations between these values obtained from animals and humans is needed to confirm their validity.

The mean values for muscarinic acetylcholine receptor occupancy and inhibitory ratio of acetylcholine-induced contraction under a steady state after oral administration of standard doses of the drugs examined were estimated to be
12.6% and 3.27%, respectively. It is known that oxybutynin and propiverine each have one pharmacologically active metabolite. However, there are no available data on the metabolite of oxybutynin. To explain why the values for muscarinic acetylcholine receptor occupancy and inhibitory ratio of acetylcholine-induced contraction of oxybutynin were low, we considered that only data regarding the unchanged drug were used in the present analysis. On the other hand, the inhibitory ratios of KCl-induced contraction varied from 0.01 to 0.48%. Further, though a significant linear relationship was found between muscarinic acetylcholine receptor occupancy and the maximum ratio of increase in bladder urinary capacity, no significant relationship was found between the ratio of KCl-induced contraction and maximum ratio of increase in bladder urinary capacity. In order to ascertain the inhibitory ratios of KCl-induced contraction, we determined the parameters of flavoxate which had only smooth muscle-relaxing effects, there are no data, however, on the inhibitory effects of KCl-induced contraction (IC50) values.

Together, these results indicate that the pharmacotherapeutic activities of the three drugs for treatment of urinary disturbance are elicited under a certain rate of muscarinic acetylcholine receptor occupancy (ca. 13%) and that the receptor occupancy of the drugs administered at standard doses is about 13%, regardless of the binding affinity to muscarinic acetylcholine receptors.

Receptor occupancy of a drug is considered to be a rational index of curative effectiveness. In the case of drug action elicited by specific receptors, it is important to clarify kinetically the relationship between drug concentration in the region of the site of action of the drug and binding of the drug to receptors. We have reported on the utility of the evaluation of the effect of dopamine D2 receptor antagonists, and 5-HT3 receptor antagonists. In this study, the predicted average receptor occupancy (ca. 13%) was lower than those antagonists. But it is reported that the receptor occupancy of oxybutynin using free plasma drug levels and binding affinity constants at the human muscarinic receptors (M2/M3) appears to be low (about 4%/25%). These values were similar to our results.

Further, our findings suggest that data obtained from animal experiments along with pharmacokinetic data obtained from preclinical studies can be used to estimate the rational therapeutic dose as well as the clinical effects of a new drug for treatment of urinary disturbance.

REFERENCES