Distigmine Bromide Produces Sustained Potentiation of Guinea-Pig Urinary Bladder Motility by Inhibiting Cholinesterase Activity

Keisuke Obara, Daisuke Chino, and Yoshio Tanaka*

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Toho University; 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan.

Received November 19, 2016; accepted March 14, 2017

Distigmine is a cholinesterase (ChE) inhibitor used for the treatment of detrusor underactivity in Japan. Distigmine’s pharmacological effects are known to be long-lasting, but the duration of its effect on urinary bladder contractile function has not been fully elucidated. The present study aimed to determine these effects in relation to the plasma concentrations of distigmine and its inhibition of ChE activities in blood, plasma, and bladder tissue. Intravesical pressures were recorded in anesthetized guinea-pigs for 12 h after the intravenous administration of saline or distigmine (0.01–0.1 mg/kg). Plasma distigmine concentrations were measured by liquid chromatograph-tandem mass spectrometry (LC-MS/MS), while ChE activities were assayed using 5,5'-dithiobis(2-nitrobenzoic acid). Distigmine (0.1 mg/kg) significantly increased the maximum intravesical pressure at micturition reflex for 12 h post-administration. In contrast, plasma distigmine was only detectable for 6 h post-administration in these animals and a one-compartment model calculated an elimination half-life of 0.7 h. However, bladder and blood acetylcholinesterase activities were significantly inhibited for 12 h after distigmine administration, although plasma ChE activities were not affected. The pharmacodynamic effects of distigmine thus persisted after its elimination from the circulation, indicating that it may bind to bladder acetylcholinesterase, producing sustained enzyme inhibition and enhancement of bladder contractility.

Key words distigmine bromide; acetylcholinesterase; guinea-pig urinary bladder; pharmacokinetics; cystometry; intravesical pressure

Distigmine bromide (distigmine) is a synthetic reversible cholinesterase (ChE) inhibitor. Its chemical structure consists of two molecules of pyridostigmine, connected by a hexamethylene structure. Clinically, this ChE inhibitor is principally used to treat Myasthenia gravis. In addition, glaucoma and underactive bladder are common indications for distigmine therapy in Japan. In particular, distigmine is one of the most important clinical urology therapeutics employed to treat lower urinary tract dysfunction and this compound is used for neurogenic underactive bladder ascribed to surgery, spinal cord injury, and diabetes. It is also effective against drug- and prostate enlargement-induced dysfunctions of urinary excretion. Distigmine thus plays a significant role as a principal therapeutic for urinary excretion dysfunctions associated with an underactive bladder, whereas new generations of anticholinergic drugs or beta3 adrenoceptor agonists tend to be employed to treat conditions associated with an overactive bladder.

Although a relatively large number of clinical reports have indicated the significant therapeutic efficacy of distigmine in urine storage disorders, there is a limited experimental evidence base relevant to its use in these conditions. In response to this, we have started to investigate the pharmacological effects of distigmine on urinary bladder contractile activity and the possible mechanisms underlying these effects. This has produced the following findings: 1) distigmine profoundly potentiates acetylcholine (ACh)-induced contractions of isolated urinary bladder tissue, without affecting basal tone; 2) distigmine increases the maximum intravesical pressure at micturition reflexes (IVPmax), without significantly affecting intravesical pressure during urine storage; and 3) distigmine does not affect urethral pressure at clinically relevant doses. In addition, we found that distigmine produced more sustained effects than another synthetic ChE inhibitor, neostigmine, and a synthetic choline ester, bethanechol, because it increased the IVPmax for more than 4 h.

Long-lasting pharmacological effects of distigmine have also been reported in relation to its anti-curare activity in rats and its inhibition of acetylcholinesterase (AChE) in human erythrocytes. However, no information about the duration of distigmine’s effects on urinary bladder motility is available. In addition, there is a lack of information on the relationships between the duration of this activity, the blood concentration of distigmine, and its inhibition of AChE.

The present study was therefore conducted to investigate the duration of the pharmacological effects of distigmine and its blood concentration. We measured the potentiation of urinary bladder motility by distigmine using cystometry and we also determined blood, plasma, and bladder ChE activities.

MATERIALS AND METHODS

Animals Female guinea-pigs (weighing 400–575 g, Sankyo Labo Service, Tokyo, Japan) were housed under controlled conditions (temperature 21–22°C, relative air humidity 50±5%, fixed 12-h light (08:00 to 20:00)/12-h dark cycle). Food and water were available ad libitum to all animals. This study was approved by the Toho University Animal Care and User Committee (approval number: 15:51-294 accredited on May 22, 2015), and conducted in accordance with the User’s Guideline to the Laboratory Animal Center of Faculty of Pharmaceutical Sciences, Toho University.

* To whom correspondence should be addressed. e-mail: yotanaka@phar.toho-u.ac.jp
Cystometry Study in Anesthetized Guinea-Pigs Changes in intravesical pressure were recorded by cystometry using a previously described method.15 All experimental surgeries, including cannula introduction and intravesical pressure measurement, were carried out under anesthesia with pentobarbital sodium (30 mg/kg intraperitoneally). When additional anesthesia was required during recordings, pentobarbital sodium was injected intravenously, paying very careful attention to the physical condition of the guinea-pig.

After anesthesia had been established, the guinea-pig was fixed in a supine position and the hair of the antebrachial, abdominal, and cervical regions was cut using an electrical hair clipper. Thereafter, tracheal and brachial/jugular vein cannulae were inserted prior to cutting the abdomen along part of the midline and exposing the urinary bladder. An injection needle (27G × 3/4; Terumo Corp., Tokyo, Japan) was inserted into a part of the urinary bladder dome with few blood vessels and nerves, and fixed with surgical tissue adhesive (Aron Alpha A; Daiichi-Sankyo Company Ltd., Tokyo, Japan). This injection needle provided a common route for intravesical injections and nerves, and fixed with surgical tissue adhesive (Aron Alpha A; Daiichi-Sankyo Company Ltd., Tokyo, Japan). The resulting blood plasma was stored at −20°C until measurement of distigmine concentrations.

Plasma distigmine concentrations were measured by Bio Medical Laboratories Inc. (Tokyo, Japan) under the following conditions. First, neostigmine bromide (20.20 µg/mL; 50 µL) as an internal standard, 2% acetic acid (20 µL), and 2-propanol (980 µL) were added to 200 µL defrosted plasma samples or 200 µL standard distigmine solutions, which were used to construct a calibration curve. The mixtures were then adequately shaken and centrifuged (4°C, 3000 rpm, 10 min). Next, 1 mL of the organic supernatant was dried under nitrogen (40°C). The dried samples were then dissolved in the HPLC mobile phase (500 µL) and subsequently centrifuged (4°C, 3000 rpm, 5 min). The supernatants were collected and centrifuged again (4°C, 10,000 rpm, 5 min). After centrifugation, 100 µL ultrapure water was added to 100 µL of each supernatant and these samples were used for the liquid chromatograph–tandem mass spectrometry (LC-MS/MS) measurement of distigmine concentrations (HPLC system: HP1100, Hewlett-Packard Company, Palo Alto, CA, U.S.A.; MS system: API4000, Applied Biosystems/MDS SCIEX).

HPLC was conducted using a TSKgel VMpak-25 column (2.0 mm internal diameter × 150 mm) at a temperature of 40°C. The sample injection volume was 10 µL and the mobile phase was composed of 30 mmol/L ammonium formate–acetonitrile (30:70, v/v), at a flow rate of 0.2 mL/min. Electrospray ionization MS employed positive polarity with a collision gas (N₂) value of 6. The curtain gas pressure was 30 p.s.i., with ion source gas 1 and 2 pressures of 30 and 80 p.s.i., respectively. The ion spray voltage was 5500 V and the temperature was 700°C. For distigmine bromide, the precursor ion m/z was 208 and the product ion m/z was 110. For neostigmine bromide, the precursor ion m/z was 223 and the product ion m/z was 208.

The standard curve for distigmine was constructed by plot-
ting the known concentration of each distigmine standard (x-axis) against the ratio of the distigmine peak area to that of neostigmine (the internal standard) on the y-axis. The resulting linear regression of the relationship between x and y, generated by the least squares method \( Y = aX + b; \text{weight} = 1/X \) was used to calculate the plasma distigmine concentrations. The measurement limit was 0.2 ng/mL.

**Measurement of Blood AChE Activity** As described in “Measurement of Plasma Distigmine Concentrations,” blood samples were collected from each guinea-pig at 3, 10, 30 min, 1, 3, 6, and 12 h after their i.v. injections. The collected bloods were treated with heparin and then used for the analysis of blood AChE activity. Blood AChE activity was determined as described previously, with minor modifications. First, the whole blood samples (5 μL) were incubated for 10 min at 37°C in a solution containing 2985 μL phosphate buffer (0.1 M, pH 7.4), 10 μL tetraisopropyl pyrophosphoramide (iso-OMPA; a selective butyrylcholinesterase (BChE) inhibitor; 3×10^{-4} μM), and 100 μL 5,5’-dithiobis(2-nitrobenzoic acid) (DTNB; 10^{-2} M). After this incubation period, 20 μL acetylthiocholine (ATCh; 7.5×10^{-3} M) was added to the solution and subsequently incubated for 3 min at 37°C, with the absorbance (Abs) measured every 30 s at 412 nm using a UV-visible spectrophotometer (Model UV-150-02; Shimadzu, Kyoto, Japan). For blank measurements, phosphate buffer was used instead of a blood sample. The rate of change of Abs (ΔAbs/min) was calculated using linear regression analysis. Finally, blood AChE activity (μmol/min/mL) was calculated using the following formula, with a molar absorbance coefficient (c) of 13600/mM/cm.

\[
\text{Blood AChE activity (μmol/min/mL)} = \Delta \text{Abs} / 13600 \times 10^3 / 5 \times 3120
\]

These blood AChE activities were expressed relative to the control value (corresponding to 100%); this was the level observed prior to i.v. administration of saline or distigmine.

**Measurement of Plasma ChE Activity** As described in “Measurement of Plasma Distigmine Concentrations,” blood samples were collected from each guinea-pig at 3, 10, 30 min, 1, 3, 6, and 12 h after their i.v. injections. The collected bloods were treated with heparin and then separated by centrifugation (4°C, 10000 rpm, 10 min). The resultant blood plasma was then used to measure plasma ChE activity.

Plasma samples (20 μL) were incubated for 10 min at 37°C in a mixture of 2980 μL phosphate buffer (0.1 M, pH 7.4) and 100 μL DTNB (10^{-2} M). These samples were not exposed to iso-OMPA (a BChE inhibitor) and their subsequent assay thus detected total ChE activity. The samples were then post-treated and assayed as described in “Measurement of Blood AChE Activity.” Plasma ChE activity (μmol/min/mL) was calculated using the following formula.

\[
\text{Plasma ChE activity (μmol/min/mL)} = \Delta \text{Abs} / 13600 \times 10^3 / 20 \times 3120
\]

The resultant activity was expressed relative to the control value (corresponding to 100%); this was the level observed prior to i.v. administration of saline or distigmine.

**Measurement of Urinary Bladder AChE Activity** At the end of the cystometry study, urinary bladder tissues were isolated from the guinea-pigs. The urinary dome area was isolated and fat tissues and the bladder trigone were removed. The isolated urinary dome was then minced into small pieces and homogenized for about 3 min using an electrical homogenizer (SM-3; Natsume Seisakusho Co., Ltd., Tokyo, Japan) in 20 mg/mL phosphate buffer (0.1 M, pH 7.4). The urinary bladder homogenates were then separated by centrifugation (4°C, 10000 rpm, 5 min) and the supernatants were used for determination of AChE activity. This was achieved by incubating supernatant samples (400 μL of 20 mg/mL) with a mixture of 2590 μL phosphate buffer (0.1 M; pH 7.4), 10 μL iso-OMPA (3×10^{-3} M), and 100 μL DTNB (10^{-2} M) for 10 min at 37°C. AChE activities were then determined using the procedure described in “Measurement of Blood AChE Activity” and bladder AChE activity was calculated using the following formula:

\[
\text{Bladder AChE activity (μmol/min/g)} = \Delta \text{Abs} / 13600 \times 10^6 / 400 \times 3120 / 20 (\text{mg/mL})
\]

**Drugs and Chemicals** The following drugs were used: distigmine bromide (Torii Pharmaceutical Cooperation Ltd., Tokyo, Japan); ATCh iodide, DTNB, and pentobarbital sodium (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan); heparin sodium (Wako Pure Chemical Industries, Ltd., Osaka, Japan); and iso-OMPA and neostigmine bromide (Sigma-Aldrich Co., St. Louis, MO, U.S.A.). All other chemicals used in the present study were commercially available and of reagent grade.

Distigmine bromide and heparin sodium were dissolved in saline. Pentobarbital sodium was dissolved in propylene glycol, ethanol-containing vehicle (ethanol–propylene glycol–0.9% NaCl; 1:4:5). ATCh and DTNB were dissolved in phosphate buffer (0.1 M, pH 7.4). All other chemicals were dissolved in distilled water.

**Statistical Analysis** Data are presented as mean values±standard error of the mean (S.E.M.). The significance of differences between mean values was evaluated by one-way ANOVA followed by Dunnett’s multiple comparison test using GraphPad Prism™ (version 6.03; GraphPad Software, San Diego, CA, U.S.A.). p values of less than 0.05 were considered to be statistically significant.

**Pharmacokinetic Analysis** The area under the plasma concentration vs. time curve (AUC) (ng·h/mL) was calculated by GraphPad Prism™ (version 6.03). Total systemic clearance \( (CL_\text{tot}) \) (mL/h/kg) was calculated using the following formula:

\[
CL_\text{tot} (\text{mL/h/kg}) = \text{dose (mg/kg)} \times 10^6 / AUC (\text{ng·h/mL})
\]

By applying a one-compartment model in GraphPad Prism®, the plasma concentrations of distigmine (C) (ng/mL) and elapsed time (t) since administration (h) were used to calculate the elimination rate constant \( (k_e) \) (h) and plasma concentration at 0 min \( (C_0) \) (ng/mL) by non-linear regression analysis using weighted least squares with reciprocal squared observation weighting. Finally, the elimination half-life \( (t_{1/2}) \) (h) and volume of distribution \( (V_d) \) (mL/kg) were calculated using the following formulas:

\[
t_{1/2} (\text{h}) = \ln 2 / k_e (\text{h})
\]

\[
V_d (\text{mL/kg}) = \text{dose (mg/kg)} \times 10^6 / C_0 (\text{ng/mL})
\]

**RESULTS**

The Effects of Distigmine on Guinea-Pig Intravesical Pressure Figure 2 shows that distigmine increased the in-
travesical pressure recorded by cystometry in the anesthetized guinea-pig. In the control animals, saline administration did not produce significant alterations of the IVP<sub>max</sub> over the 12-h recording period (Fig. 2A). The time-course of changes in urinary bladder motility are shown in Fig. 3. Perhaps due to the unavoidable effects of recording for 12h, such a gradual decrease in the general condition of the animal under anesthesia, the IVP<sub>min</sub> gradually declined in saline-injected guinea-pigs (Fig. 3B), while the urination interval and volumes gradually increased over time (Figs. 3C, D).

Figures 2B–D illustrate representative traces showing the changes in guinea-pig intravesical pressure following i.v. administration of the indicated doses of distigmine. IVP<sub>max</sub> was increased after distigmine administration in a dose-dependent manner (Fig. 3A). At a dose of 0.01 mg/kg, the increase in IVP<sub>max</sub> was sustained for 4–5h after distigmine administration, while doses of 0.03 and 0.1 mg/kg produced significant potentiation of IVP<sub>max</sub> for more than 12h (Fig. 3A).

The decrease in IVP<sub>min</sub> (Fig. 3B), increase in urination interval (Fig. 3C), and increase in urine volume (Fig. 3D) observed over time in saline-injected control guinea-pigs were smaller or absent in guinea-pigs treated with distigmine (Figs. 3B–D). Although no statistically significant differences were observed, the post-voiding residual urine volume at the end of the 12-h intravesical pressure recording period was lower in animals treated with 0.03 or 0.1 mg/kg distigmine (1.80±0.58 g for saline; 1.70±0.46 g for 0.01 mg/kg; 0.70±0.09 g for 0.03 mg/kg; 0.64±0.20 g for 0.1 mg/kg).

**Plasma Concentrations of Distigmine**

Figure 4A shows the plasma concentrations of distigmine over the 12-h period following the i.v. administration of saline or distigmine (0.01, 0.03, 0.1 mg/kg). Figure 4B shows the non-linear regression

[Figures 2 and 3 are described in the text.]
mine dose-dependent manner. In contrast, total AChE activities were measured in the presence of iso-OMPA (10⁻⁶ mol/L), a selective BChE inhibitor. The inhibitory effects of distigmine increased in a dose-dependent manner and reached their maximum levels 1–3 h after administration. The inhibitory effects of distigmine on AChE activity were sustained during the measurement period and statistically significant effects were observed for 12 h after the administration of all tested doses (Fig. 5A).

Figure 5B shows the time–course of plasma ChE activities in guinea-pigs that received 0.01, 0.03, or 0.1 mg/kg i.v. distigmine. Although this activity was slightly reduced in distigmine-treated animals, the effects were neither dose-dependent nor statistically significant.

Figure 6 shows the AChE activities of the bladder tissues isolated from guinea-pigs treated with 0.01, 0.03, or 0.1 mg/kg distigmine i.v. according to the protocol shown in Fig. 2. Even 12 h after distigmine administration, these AChE activities were lower than those of saline-injected guinea-pigs; this enzyme inhibition was distigmine dose-dependent. Although 0.01 mg/kg distigmine did not produce a statistically significant effect, the AChE inhibition induced by 0.03 and 0.1 mg/kg was statistically significant.

DISCUSSION

The present study aimed to elucidate the relationship between the duration of the effects of distigmine on urinary bladder contractile activity, ChE inhibition, and plasma distigmine levels.

Our findings demonstrated that distigmine had sustained effects on bladder emptying. The IVF_max was significantly increased for at least 12 h in guinea-pigs treated with 0.03 or 0.1 mg/kg distigmine (Figs. 2, 3A); the post-voiding residual urine volume was also lower in these animals, although this difference was not statistically significant in this study. Our previous study showed that distigmine potentiated urinary bladder contractile function in the anesthetized guinea-pig for at least 4 h. Our present study confirms the previous findings and extends them by showing that these effects of distigmine were much longer lasting than we had previously anticipated. Consistent with our present finding, Herzfeld et al. reported that distigmine showed sustained anti-curare activity in rats.

With respect to enzyme activity, distigmine was reported to produce long-lasting inhibition of erythrocyte AChE. The present study also provided evidence that distigmine (0.03–0.1 mg/kg) inhibited blood and bladder AChE activities for at least 12 h (Figs. 5A, 6). Since brain AChE activity was not inhibited by distigmine, its pharmacological actions are presumed to be mediated by effects on peripheral AChE. Therefore, the present findings and previous reports indicate that the observed sustained effects of distigmine on urinary bladder contractile function are mediated by its inhibition of peripheral AChE.
bladder motility reflect its inhibition of bladder tissue AChE. The present biochemical AChE activity data also strongly support our previous suggestion that the potentiation of urinary bladder contraction by distigmine reflects increases in the concentrations of ACh in the synaptic cleft between parasympathetic nerve terminals and urinary bladder smooth muscle cells.\(^{14}\)

We also examined the relationship between the effects of distigmine on urinary bladder function and its plasma concentration, determined by LC-MS/MS. This analysis produced the surprising finding that although distigmine produced pharmacodynamic effects that lasted for 12 h, most distigmine was rapidly eliminated from the blood following i.v. injection; as compared to the plasma level 3 min after injection, about 95% had been eliminated from the plasma by 3 h, and about 99% after 6 h. These findings suggest that the pharmacological effects of distigmine were not significantly correlated with its plasma levels. Itô et al. also reported that rat erythrocyte AChE inhibition by distigmine did not correlate with the plasma concentrations of this drug following oral administration.\(^{22}\) The possibility that active metabolites of distigmine generated by hepatic metabolism contribute to these pharmacodynamic effects cannot be completely ruled out at present, although this seems unlikely because: 1) distigmine was reported to be excreted by the kidneys\(^{24}\) using \(^{125}\)C-labeled distigmine; 2) three chemical candidates that are predicted to be generated by decomposition of distigmine did not affect AChE activity\(^{25}\); 3) distigmine-mediated enhancement of ACh-induced contraction of guinea pig urinary bladder smooth muscle lasted for \(\geq 12\) h after washing out with distigmine-free solution\(^{25}\); and 4) distigmine inhibited recombinant human AChE activities for \(>48\) h after the separation of distigmine from the enzyme by centrifugation (our unpublished observation). One possible explanation for this discrepancy would thus be the retention of distigmine within urinary bladder tissues or a robust binding to AChE, which would sequester the compound at its site of action. However, we do not have any conclusive experimental evidence of this and further investigations are required to explore the mechanisms underlying these findings.

The present study of post-voiding residual urine volume indicated that this was lower in guinea-pigs treated with 0.03 or 0.1 mg/kg distigmine, although this effect was not statistically significant. This finding was in accordance with previous reports showing that distigmine reduced post-voiding residual urine volume in humans.\(^{4,5}\) The present study found that control guinea-pigs, which received saline injections, showed a time-dependent decrease in IVP\(_{\text{min}}\), a prolongation of urination interval, and an increase in urination volume (Figs. 3B–D). A plausible explanation for these changes in urinary bladder voiding functions would be that prolonged saline infusion into the anesthetized guinea-pig overloaded the bladder and thus produced pseudo-underactive bladder. Since these impairments were not observed in the guinea-pigs that received distigmine, this compound might protect from the functional attenuation associated with prolonged saline infusion into the urinary bladder.

Our investigation of the effects of 0.01 mg/kg distigmine
showed that, similar to 0.03 and 0.1 mg/kg, this dose inhibited blood and urinary bladder tissue AChE activities 12 h after i.v. administration, although the latter effect was not statistically significant; in addition, the effects of this lower dose on $\text{IP}_{\text{max}}$ only lasted for 4–5 h. The inhibitory effects of 0.01 mg/kg distigmine on AChE activities may not be sufficient to generate 12-h effects on the urinary bladder. Another explanation for this observation relates to the involvement of inhibitory autoreceptors. Similar to other neurotransmitter releasing systems, muscarinic autoreceptors are suggested to be present at the parasympathetic nerve terminals, where their stimulation inhibits ACh release. For instance, stimulation of the M4 muscarinic receptor is suggested to induce feedback and reduce ACh release from the nerve terminals in the mouse urinary bladder.26,27 Administration of 0.01 mg/kg distigmine may therefore have elevated ACh concentrations and thus stimulated autoreceptors at parasympathetic nerve terminals; this could have inhibited ACh release from the nerve terminals and limited the potentiation of urinary bladder voiding. Since 0.03 and 0.1 mg/kg distigmine exerted sustained 12-h effects on urinary bladder function, this level of distigmine may be required to overcome the attenuation of pharmacological effects by autoinhibition of ACh release.

The present study found that although distigmine slightly suppressed plasma ChE activities, these effects were neither dose-dependent nor statistically significant (Fig. 5B). Naik et al. reported that more than 90% of plasma ChE activity arises from BChE in Hartley guinea-pig plasma and that this situation is similar in humans.28,29 Therefore, any observed plasma ChE inhibitory effects are thought to reflect effects on BChE. Our present findings were consistent with a report by Yamamoto et al., which suggested that the inhibitory effects of distigmine on plasma ChE activities were independent of the dose administered.29 On the other hand, very strong decreases in plasma ChE activities have been reported in clinical cases of distigmine-evoked cholinergic crisis, which is the most important side effect of this drug.30–32 However, if our guinea-pig data can be applied to humans, they indicate that clinically-employed doses of distigmine should not induce a severe cholinergic crisis.

CONCLUSION

In summary, the carbamate ChE inhibitor, distigmine, produced a strong and sustained potentiation of urinary bladder motility, even after the compound had been eliminated from the circulation, indicating the possibility that the pharmacological actions of distigmine on the lower urinary tract do not correlate with its plasma concentration. The long-lasting effects of distigmine on the urinary bladder seem to be fundamentally mediated by its inhibition of AChE activity in this smooth muscle tissue. Distigmine might selectively but rigidly bind to urinary bladder tissue AChE in a pseudo-reversible manner and thus exert long-lasting effects on urinary bladder emptying.

Acknowledgment Distigmine bromide was provided free of charge by Torii Pharmaceutical Co., Ltd. (Tokyo, Japan).

Conflict of Interest Torii Pharmaceutical Co., Ltd. (Tokyo, Japan) funded the measurement of plasma distigmine concentrations.

REFERENCES

12) Tsuno N, Miyata H, Nakayama K. Dysuria induced by psychotrophic drugs—Appropriate usage of distigmine bromide (Ubretid), therapeutic medicine of dysuria—. No to Seishin no Igaku (Brain Science and Mental Disorders), 14, 149–153 (2003).


