Sustainable Effects of Distigmine Bromide on Urinary Bladder Contractile Function

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Keywords

Distigmine bromide · Acetylcholinesterase inhibitor · Urinary bladder · Cystometry · Blood concentration

Abstract

Distigmine bromide (distigmine) is a reversible carbamate cholinesterase (ChE) inhibitor that is used to treat myasthenia gravis. In Japan, it is also used as a remedy for urination disorder (underactive bladder). The most distinctive pharmacological feature of distigmine is its long-lasting action compared to that of other ChE inhibitors. In animals and humans, distigmine was reported to inhibit acetylcholinesterase (AChE) and improve myasthenia gravis for an extended period. Few studies have examined the sustainability of this enhancing effect on the contractile function of urinary bladder smooth muscle. In addition, the cause of this long-lasting feature remains unclear. In this review, we present our findings for the long-lasting feature of distigmine on isolated urinary bladder contraction and in vivo urinary function of guinea pig. We also present our results on the mechanism of its long-lasting sustainability using recombinant human AChE.

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Introduction

Distigmine bromide (Distigmine) is a carbamate-type cholinesterase (ChE) inhibitor with a chemical structure containing 2 molecules of pyridostigmine also classified

as carbamate-type ChE inhibitors, and a hexamethylene group (Fig. 1). Similar to other ChE inhibitors such as pyridostigmine, neostigmine, and ambenonium, the main indication for the use of distigmine is myasthenia gravis (Fig. 1) [1–3]. Distigmine has been clinically used in Japan for the treatment of urinary dysfunction (underactive bladder) [4–8] and is also reported to be used in European countries (the United Kingdom [9–11] and Greece [12, 13]).

The therapeutic effects of distigmine against urinary dysfunction have generally been inferred to be due to its enhancement of acetylcholine (ACh)-mediated contraction of urinary bladder smooth muscle (UBSM), which is mainly elicited via the muscarinic M_3 receptor [14]. However, although many results of its clinical usage have been reported, its pharmacological actions and underlying mechanisms have not been extensively studied in its target tissue (UBSM) even on a basic level with animals. Therefore, we performed basic in vitro and in vivo researches and have clarified the pharmacological effects of distigmine using UBSM isolated from guinea pig and anesthetized whole animals as described below. Guinea pigs are often used as research models for the human urinary tract as the guinea pig's lower urinary tract anatomy and urodynamic profile of micturition are similar to those of humans [15]. From this series of experiments, we found that (1) distigmine strongly potentiates ACh-induced contraction of the isolated UBSM preparations [16]; (2) distigmine significantly increases intravesical pressure (IVP) during urination reflex without substan-

Fig. 1. Structures of the reversible carbamate ChE inhibitors (distigmine, neostigmine, pyridostigmine, and ambenonium).

tially affecting pressure during micturition reflex voiding and exhibits minor effects on IVP during urine accumulation (compared to the time before distigmine administration) [16, 17]; (3) the increasing effects of distigmine on IVP during urination reflex is caused by the augmentation of UBSM contraction and does not involve urethral pressure increase [18]; (4) distigmine significantly increases bladder contractile force only at the time of micturition reflex (the maximum IVP at the time of voiding; IVP_{max}) without affecting bladder volume, and this effect lasts for >4 h (compared to before distigmine administration) [19]. Based on these points, distigmine might be superior to neostigmine or bethanechol (a synthetic choline ester) as a therapeutic medicine for the treatment of underactive bladder [19, 20]. As mentioned above, our research group clarified the pharmacological effects of distigmine on both isolated UBSM and in situ bladder preparations, and revealed its rather long-lasting effects.

This long-lasting characteristic is the most outstanding of its pharmacological action when compared to other ChE inhibitors. The sustainability of the pharmacological actions of distigmine has been reported based on its acetylcholinesterase (AChE) inhibitory action and therapeutic effect against myasthenia gravis: (1) In rats intraperitoneally administered distigmine, anti-curare action (antagonistic action against d-tubocurarine's muscle relaxant action) was observed up to 24 h after administration [21]; (2) In rats orally administered distigmine, AChE activities in erythrocytes, colon, bladder, and submandibular gland remained suppressed even 12 h following administration [22]; (3) oral administration of distigmine to myasthenia gravis patients caused symptom re-

lief for 36 h [1]; (4) The estimated recovery half-life value was ~40 h based on distigmine's inhibitory effect on AChE activity in erythrocytes from humans [23]. Conversely, the persistence of the enhancing effects of distigmine on the contractile force of UBSM has not been reported elsewhere. Moreover, the cause of the long-lasting feature of distigmine has not been elucidated. In this review, we describe the long-lasting characteristics of distigmine found with isolated UBSM or in situ UB preparations in anesthetized animals. We also describe the results of studies carried out to elucidate its underlying mechanism using recombinant human AChE (rhAChE).

Effects of Distigmine on Guinea Pig Urinary Bladder Function and Relationship between these Effects and Blood Concentration

Changes in IVP in anesthetized guinea pigs assessed using the cystometry method revealed that distigmine continuously increased IVP_{max} over 4 h, while neostigmine transiently increased IVP_{max} [19, 21]. However, whether the duration of distigmine's increasing effects on UB motility could last >4 h is yet to be examined. The relationship between the sustained enhancing effect of distigmine on IVP_{max} and its pharmacokinetics, particularly its plasma concentration, and the inhibitory action on AChE activity have not been examined. Therefore, we used the cystometry method to answer these questions as presented below [24].

Figure 2a is a typical experimental trace showing the anesthetized guinea pigs' IVP changes that were mea-

Fig. 2. Effects of distigmine bromide on guinea pig IVP and blood AChE activity, and the time-course for plasma distigmine bromide concentration. **a** Representative pressure trace showing the urinary bladder contractions associated with micturition. When 3 stable contractions were observed, distigmine bromide (0.03 mg/kg) was administered i.v., and urinary reflexes were observed over 12 h. **b** Summarized data for the effects of distigmine bromide (0.01–0.1 mg/kg) and saline on the IVP_{max} changes recorded following the protocol shown in (a). * $p < 0.05$, ** $p < 0.01$ versus saline (one-way analysis of variance followed by Dunnett's multiple

comparison test). **c** Time-course for the blood AChE activity measured in the presence of 10^{-6} mol/L iso-OMPA. * $p < 0.05$, ** $p < 0.01$ versus saline (one-way analysis of variance followed by Dunnett's multiple comparison test). **d** Time-course of the plasma distigmine bromide concentration measured by LC-MS/MS. Data represent the mean values $\pm$ SEM ($n = 4$). These data were reproduced with permission from Obara et al. [24]. iso-OMPA, tetraisopropyl pyrophosphoramidate; IVP_{max} , maximum intravesical pressure; AChE, acetylcholinesterase.

sured by cystometry over 12 h after distigmine administration (0.03 mg/kg, i.v.). Figure 2b is the quantitative analysis results of the recordings when physiological saline or distigmine (0.01, 0.03, 0.1 mg/kg) was administered to guinea pigs. In Figure 2b, changes in IVP_{max} (100% = IVP_{max} before distigmine administration) are shown up to 12 h after the administration of saline or each dose of distigmine. As shown in Figure 2a and b, the increase in IVP_{max} was more pronounced at a dose of 0.03 mg/kg than at 0.01 mg/kg, and this value remained high until 12 h after distigmine administration. At a dose of 0.1 mg/kg, the increase in IVP_{max} was more pronounced with a statistically significant difference 12 h af-

ter distigmine administration. To our knowledge, this is the first finding to demonstrate that distigmine also exhibits long-lasting properties for at least 12 h for its potentiating effects on UBSM motility, thereby supporting its long-lasting anti-curare activity [21] and anti-AChE activity in erythrocyte [23]. Figure 2c shows the effects of distigmine (0.01–0.1 mg/kg) on guinea pig blood AChE activity examined over 12 h following the administration of distigmine. In this series of experiments, blood samples were collected from guinea pigs when IVP and AChE activity were measured by the 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) method in the presence of tetraisopropyl pyrophosphoramidate (10^{-6} mol/L, an inhibitor of bu-

tyrilycholinesterase [BChE]) to eliminate its influence. Distigmine was found to strongly inhibit blood AChE activity depending on its dose. AChE activity was at its lowest at 1–3 h after distigmine administration and was continuously suppressed by any distigmine doses, with statistically significant differences observed even 12 h after distigmine administration relative to the control group (saline-treated group). Furthermore, AChE activity of the bladder tissues isolated 12 h after administration of any distigmine dose (0.03 and 0.1 mg/kg) was found to be significantly lower than that found in the saline group (data not shown). In contrast, distigmine had no significant effect on plasma ChE (BChE) activity (data not shown). These findings suggest that distigmine's enhancing effect on UB motility (the increasing effect on IVP_{max}) strongly reflects the ChE inhibitor-induced suppression of UB AChE activity.

We proceeded to determine whether the potentiating effect of distigmine on UB motility is based on its plasma concentration. The time-course change of distigmine's plasma concentration was measured by LC-MS/MS using plasma obtained from blood collected from guinea pig when IVP was measured by cystometry. As shown in Figure 2d, administering distigmine (0.01–0.1 mg/kg, i.v.) increased its plasma concentrations at 3 min to 38.6 ± 0.93 ng/mL (0.01 mg/kg), 113.8 ± 7.58 ng/mL (0.03 mg/kg), and 451 ± 29.4 ng/mL (0.1 mg/kg). In contrast, at 3 h after the administration, these concentrations fell to 1.93 ± 0.34 ng/mL (0.01 mg/kg), 6.30 ± 1.10 ng/mL (0.03 mg/kg), and 25.3 ± 12.58 ng/mL (0.1 mg/kg), demonstrating that 95% of distigmine disappeared from plasma compared to 3 min post-administration. Furthermore, at 6 h after the administration, these concentrations reduced to 0.40 ± 0.12 ng/mL (0.01 mg/kg), 1.40 ± 0.46 ng/mL (0.03 mg/kg), and 5.37 ± 4.01 ng/mL (0.1 mg/kg), depicting that 99% of distigmine disappeared from plasma compared to the concentrations 3 min post-administration. These results indicate a very significant feature of distigmine's pharmacology; the enhancing effect of distigmine on UB contractile function and its inhibitory effect on AChE activity are independent of its plasma concentration. That is, the long-lasting characteristic distigmine-induced potentiation of UB contraction and inhibition of AChE activity could be produced without its supply from plasma. In fact, Ito et al. [22] reported that the dissociation occurs between distigmine-induced inhibition of erythrocyte AChE activity and its plasma concentration in rats when orally administered. Furthermore, a plausible contribution by distigmine's metabolites on its long-lasting pharmacological effects can be

ruled out, as this ChE inhibitor is excreted via the renal system [25].

From these findings and reports, the following hypotheses might explain the long lasting potentiating/inhibitory effects exhibited by distigmine on UB contractile function/AChE activity without a dependence on its plasma concentration: distigmine may remain in the UBSM tissue for an extended time or bind tightly to AChE in the UB tissue (i.e., it is difficult to dissociate from AChE).

Sustainability of the Enhancement Effects of Distigmine on ACh-Induced Contraction of UBSM

In the previous chapter, we reported that distigmine produces long-lasting enhancement of UB contractile function and inhibition of AChE activity even after elimination from plasma. This is either because it remains in the UB tissue for an extended time, including the plasma membrane of UBSM cells, or it forms a stable complex by tightly binding to AChE. With these possibilities, studies using isolated UBSM tissues appear to be effective in elucidating the factors that cause distigmine to increase UB contraction and inhibit AChE for a long time. This is because studies using the isolated UBSM tissues can eliminate the influence of distigmine present in the blood, and to an extent, the influence of its potential metabolites. In this chapter, we present the results obtained using isolated guinea pig UBSM tissues [26]. In this series of experiments, we determined the length of time that distigmine could demonstrate its enhancing/inhibitory effects on ACh-induced contraction and ChE activity of UB tissue after removal of distigmine from the nutrient solution (after distigmine wash-out). We have also discussed the results of comparative studies on the sustainability of pharmacological actions of other carbamate-type ChE inhibitors (i.e., pyridostigmine, neostigmine, ambenonium; Fig. 1).

Figure 3a is a representative trace of the potentiating effect of distigmine (10^{-6} mol/L) on ACh (3×10^{-6} mol/L)-induced contraction of guinea pig UBSM and its sustainability up to 12 h after distigmine wash-out. Figure 3b shows the sustainability of the potentiating effects of other ChE inhibitors (pyridostigmine, neostigmine, and ambenonium, 10^{-6} mol/L) together with the results for distigmine shown in Figure 3a.

For each recording, the changes in ACh-induced contraction after inhibitor treatment/wash-out were recorded up to 12 h after inhibitor wash-out for contraction by 3×10^{-6} mol/L ACh (contraction before inhibitor admin-

Fig. 3. Effects of distigmine, neostigmine, pyridostigmine, and ambenonium on 3×10^{-6} mol/L ACh-induced contraction and ChE activity in guinea pig UBSM up to 12 h after w/o. **a** A representative trace of the enhancing effects of distigmine (10^{-6} mol/L) on 3×10^{-6} mol/L ACh-induced contraction up to 12 h after w/o. Arrow: administration of 3×10^{-6} mol/L ACh; w: w/o. **b** Summarized data for the effects of distigmine, neostigmine, pyridostigmine,

and ambenonium on 3×10^{-6} mol/L ACh-induced contraction up to 12 h after w/o of each inhibitor, following the protocol shown in (a). **c** Inhibiting effects of distigmine, neostigmine, pyridostigmine, and ambenonium on ChE Activity up to 12 h after w/o of each drug. Data represent the mean values $\pm$ SEM ($n = 4$). These data were reproduced with permission from Obara et al. [26]. ACh, acetylcholine; ChE, cholinesterase; w/o, washout.

istration being 100%). All ChE inhibitors (distigmine, neostigmine, pyridostigmine, ambenonium: 10^{-6} mol/L) including distigmine significantly enhanced ACh-induced contraction when administered 30 min before ACh (compared to ACh-induced contraction before ChE inhibitor treatment). However, the potentiating effects of the 3 ChE inhibitors (neostigmine, pyridostigmine, ambenonium) disappeared within 2–3 h after their wash-out (Fig. 3b). In contrast, potentiation of ACh-induced contraction by distigmine remained significant until 12 h after wash-out (Fig. 3a, b). Figure 3c shows the effects of various ChE inhibitors on the ChE activity of guinea pig UBSM tissues measured by the DTNB method for 12 h after their wash-out. All ChE inhibitors (distigmine, neo-

stigmine, pyridostigmine, ambenonium: 10^{-6} mol/L each) including distigmine significantly inhibited the ChE activity of UBSM tissues when administered 30 min before activity measurement (compared to ChE activity before ChE inhibitor treatment). However, the inhibitory actions of 3 ChE inhibitors (neostigmine, pyridostigmine, and ambenonium) were restored in a time-dependent manner after their wash-out. In contrast, distigmine significantly inhibited ChE activity of UBSM tissues up to 12 h after its wash-out (compared to ChE activity before distigmine treatment). These findings indicate that its retention in blood for an extended time cannot clarify why it exhibits a remarkable long-lasting pharmacological effect. These findings also suggest that distigmine binds

Fig. 4. Effects of distigmine degradation products (Compounds A–C; 10^{-3} mol/L) on ChE activity in guinea pig UBSM up to 12 h after w/o. Data represent the mean values $\pm$ SEM ($n = 3$). These data were reproduced with permission from Obara et al. [26]. ChE, cholinesterase; w/o, washout.

tightly to AChE to produce a distigmine-AChE complex that is difficult to dissociate.

In the next series of experiments, we sought to determine whether the long-lasting inhibitory effects of distigmine on ChE activity were produced by distigmine itself or its degradation products. Hence, the inhibitory effects on ChE activity were investigated for the candidate substances (Fig. 4; degradation products), which might be generated by distigmine's degradation by ChE. Based on its chemical structure, distigmine contains 2 pyridostigmine molecules linked via a hexamethylene group, thereby containing 2 carbamoyl groups that can bind to ChE (Fig. 1, 4). A candidate of distigmine's degradation product with a structure containing one carbamoyl group may therefore inhibit ChE activity. Some products that do not have a carbamoyl group have been reported to show inhibitory action against ChE activity by acting at non-enzyme active sites (active site: serine residue) [27]. Based on these chemical-based discussion and report, it is plausible that distigmine's degradation products that dissociated from ChE after degradation may inhibit its enzyme activity for an extended time by binding to ChE. However, the candidates of distigmine's decomposition products (Compounds A, B, and C) did not inhibit ChE activity (Fig. 4).

Therefore, the possibility that any degradation product of distigmine that dissociated from ChE could bind to this enzyme, leading to inhibition, was ruled out. Interestingly, Compound C, a decomposition candidate that loses one carbamoyl group bonded to the aromatic ring structure but has a carbamoyl group that could bind to ChE and inhibit its activity, did not inhibit ChE activity (Fig. 4). This finding suggests that the presence of both terminal aromatic ring structures is essential for distigmine to exert its ChE inhibitory action, and may closely relate to the long-lasting property of distigmine. As deletion of one of the aromatic rings causes the π - π interaction between the terminal aromatic ring structures to disappear, ultimately changing the steric structure, this may serve as a possible molecular mechanism underlying this finding. Consequently, the binding ability of the carbamoyl group of distigmine to an active site (serine residue) of ChE might be lost. A compound with 2 molecules of neostigmine (Fig. 1) bonded via a long-chain methylene group was also reported to improve the symptoms in myasthenia gravis patients for an extended time when orally administered [1].

To briefly summarize, in this chapter, we revealed that the potentiating effect of distigmine on ACh-induced UBSM contraction and ChE inhibiting effect lasts for

12 h, even after it is washed-out. This effect is also distinguishably longer than the effect of the actions of other ChE inhibitors. A plausible mechanism to explain this feature of distigmine is that the binding of distigmine to ChE results in a very stable distigmine-ChE complex, which makes it very difficult for distigmine to dissociate from the enzyme (ChE). Both molecules of pyridostigmine were also found to be necessary for distigmine to exhibit its long-lasting pharmacological actions.

Sustainability of the Inhibitory Effects of Distigmine on rhAChE

Two plausible mechanisms may be used to explain the very long-lasting potentiating action exhibited by distigmine against UBSM contractile functions: (1) distigmine tightly binds to ChE (AChE) to produce a chemically stable distigmine-AChE complex, and (2) distigmine remains in the plasma membrane and is supplied to AChE for an extended time. To examine the former possibility, we measured the ChE activity of the isolated UBSM tissues and found that distigmine continues to inhibit ChE for at least 12 h after wash-out. However, this result was also obtained by studies using UBSM tissues, and thus, the latter possibility that “distigmine’s stays in the plasma membrane for an extended time” cannot be completely excluded. Therefore, we attempted to present more direct evidence that distigmine acts directly on AChE to inhibit its enzymatic activity for an extended time by performing more molecular level analyses via biochemical studies using rhAChE isolated from cells. The results are described below [28].

First, we briefly describe the method used to measure the dissociation rate constant of various ChE inhibitors against rhAChE. In this series of experiments, rhAChE and unreacted ChE inhibitors were separated after treatment with ChE inhibitors by ultrafiltration with a Nanosep® 10K centrifuge device. By using a centrifuge for ultrafiltration, AChE remained on the filtration membrane, while the unreacted ChE inhibitors were transported to the filtrate. Therefore, the binding reaction could be terminated with a selectively advanced dissociation reaction, where the dissociation rate constant for each ChE inhibitor could be calculated.

Figure 5a displays the results obtained using the method described above and shows the activity of rhAChE after treatment with 4 ChE inhibitors (distigmine, pyridostigmine, neostigmine, and ambenonium) and the time course of rhAChE activity up to 6 h after the wash-out of these ChE inhibitors. AChE activity was lowered to

$17.2 \pm 3.4\%$ by distigmine (10^{-6} mol/L); $35.1 \pm 5.3\%$ by pyridostigmine (10^{-6} mol/L); $8.4 \pm 3.6\%$ by neostigmine (10^{-6} mol/L); and $2.0 \pm 0.5\%$ by ambenonium (10^{-6} mol/L each) with AChE activity in a control device without ChE inhibitor as 100%. When treated with pyridostigmine, neostigmine, and ambenonium, the inhibited AChE activity gradually recovered after the wash-out of each ChE inhibitor and returned to the control device’s AChE activity level within 2–4 h. The dissociation rate constants to rhAChE of these ChE inhibitors except distigmine were $0.51 \pm 0.05 \text{ h}^{-1}$ (pyridostigmine), $0.66 \pm 0.03 \text{ h}^{-1}$ (neostigmine) and $1.41 \pm 0.08 \text{ h}^{-1}$ (ambenonium). The dissociation half-lives were estimated from the dissociation rate constants: 1.36 h (pyridostigmine), 1.05 h (neostigmine), and 0.49 h (ambenonium).

On the other hand, when treated with distigmine, the inhibited AChE activity was hardly recovered after wash-out and was only recovered to $24.4 \pm 7.4\%$ (0 min: $17.2 \pm 3.4\%$) after 6 h (Fig. 5a). Furthermore, compared to the activity of rhAChE when treated with other ChE inhibitors, its activity with distigmine remained significantly reduced for 6 h after its wash-out ($p < 0.01$; compared to other ChE inhibitor treatment groups).

Figure 5c displays the results for the sustainability of the inhibitory effects of distigmine over an extended period; inhibitory effects of distigmine and pyridostigmine against rhAChE activity were determined and changes in AChE activity due to suppression were investigated up to 48 h after wash-out. Treatment with these ChE inhibitors (10^{-6} mol/L each) reduced the AChE activity to $17.1 \pm 1.4\%$ (distigmine) and $30.9 \pm 1.8\%$ (pyridostigmine). The inhibited activity of rhAChE treated with pyridostigmine gradually recovered after wash-out, and completely recovered after 6 h as depicted in Figure 5a. The inhibited activity of rhAChE treatment with distigmine was restored to $49.2 \pm 6.6\%$ at 48 h after wash-out, but the activity remained significantly inhibited compared to pyridostigmine-treated rhAChE ($p < 0.01$). The dissociation rate constant of distigmine for rhAChE was tentatively calculated as $0.012 \pm 0.001 \text{ h}^{-1}$ (Fig. 5d), and the dissociation half-life determined from the dissociation rate constant was 57.8 h.

These results suggest that distigmine acts directly on AChE for extended inhibition of its enzymatic activity and could dissociate (very slowly) from AChE compared to other ChE inhibitors. This pharmacological feature might be due to the long chain alkyl groups in its structure (Fig. 1). In fact, Perola et al. [29] reported an interesting study result. The dissociation rate constants of substances obtained by introducing a long-chain alkyl group into

Fig. 5. Time-course for the changes in rhAChE activity after treatment with distigmine, pyridostigmine, neostigmine, and ambenonium and subsequent w/o of the drugs. **a** Time-course for the changes in rhAChE activity after treatment with each ChE inhibitor (each 10^{-6} mol/L) for 6 h after inhibitor w/o. Data represent the mean values $\pm$ SEM ($n = 4$, except pyridostigmine, where $n = 5$). ** $p < 0.01$, distigmine versus other ChE inhibitors 6 h after w/o (two-way analysis of variance followed by Tukey's multiple comparison test). **b** The plot used to obtain the dissociation rate constant of each ChE inhibitor shown in (a). **c** Time-course for the changes in rhAChE activity after treatment with distigmine and pyridostigmine (each 10^{-6} mol/L) until 48 h after w/o. Data represent the mean values $\pm$ SEM ($n = 3$). ** $p < 0.01$ at 48 h after w/o (two-way analysis of variance followed by Tukey's multiple comparison test). **d** The plot used to obtain the dissociation rate constant of distigmine shown in (c). These data were reproduced with permission from Obara et al. [28]. AChE, acetylcholinesterase; ChE, cholinesterase; w/o, washout.

physostigmine, which was first identified as a ChE inhibitor, were smaller compared to that of physostigmine itself. Perola et al. [29] thought that by introducing long-chain alkyl groups into these physostigmine derivatives, the water molecules needed to drive decarbamylation could not reach the active site of AChE. If this speculation is applied to distigmine, hexamethylene groups introduced into distigmine block the access of water molecules needed for the disassociation reaction from AChE. Thus, distigmine is 40- to 120-fold less likely to dissociate from AChE compared to other ChE inhibitors. On the contrary, the apparent affinity of various ChE inhibitors for AChE was ranked as follows based on their pIC_{50} values:

ambenonium > neostigmine > distigmine > pyridostigmine (data not shown). Therefore, distigmine having a higher affinity for AChE than other ChE inhibitors may be ruled out as the reason for the smaller dissociation rate constant of distigmine for AChE than that of other ChE inhibitors.

Conclusions

Distigmine exhibits both a potentiating effect on UBSM contractile function as well as an inhibiting effect on UB AChE for an extended period after its disappear-

Fig. 6. A schematic summary of the molecular mechanism of distigmine. AChE, acetylcholinesterase; ACh, acetylcholine; UBSM, urinary bladder smooth muscle.

ance from blood. These distinguishable long-lasting pharmacological actions are due to the extreme difficulty of dissociating distigmine from AChE. This property is one of the mechanisms underlying the prolonging of the enhancing UB motility when used as an underactive bladder therapeutic agent (Fig. 6).

References

- Pateisky K. [Treatment of myasthenia gravis pseudoparalytica; clinical testing and therapy with BC substances (BC40, BC47, BC48 and BC 51)]. *Wien Z Nervenheilkd Grenzgeb.* 1959;17:53–84.
- Emeryk E, Wasowicz B. Evaluation of “Ubretid” (BC 51) in the treatment of myasthenia gravis. *Neurol Neurochir Psychiatr Pol.* 1963 Sep-Oct;13:611–7.
- Sweetman SC. *Martindale: the complete drug reference*. 35th ed. London, the United Kingdom: Pharmaceutical Press; 2007. p. 573.
- Oka N, Hasegawa S. [Treatment of dysuria with anticholinesterases: ubretid]. *Hinyokika Kyo.* 1967 Jan;13(1):42–51.
- Tanaka Y, Masumori N, Itoh N, Furuya S, Nishizawa O, Tsukamoto T. Symptomatic and urodynamic improvement by oral distigmine bromide in poor voiders after transurethral resection of the prostate. *Urology.* 2001 Feb;57(2):270–4.
- Yamanishi T, Yasuda K, Kamai T, Tsujii T, Sakakibara R, Uchiyama T, et al. Combination of a cholinergic drug and an alpha-blocker is more effective than monotherapy for the treatment of voiding difficulty in patients with underactive detrusor. *Int J Urol.* 2004 Feb;11(2):88–96.
- Tanaka Y, Masumori N, Tsukamoto T, Furuya S, Furuya R, Ogura H. Treatment strategy according to findings on pressure-flow study for women with decreased urinary flow rate. *Adv Urol.* 2009;2009:782985.
- Sugaya K, Kadekawa K, Onaga T, Ashitomi K, Mukouyama H, Nakasone K, et al. [Effect of distigmine at 5 mg daily in patients with detrusor underactivity]. *Nippon Hinyokika Gakkai Zasshi.* 2014 Jan;105(1):10–6.
- Cameron MD. Distigmine bromide (ubretid) in the prevention of postoperative retention of urine. *J Obstet Gynaecol Br Commonw.* 1966 Oct;73(5):847–8.
- Smith PH, Cook JB, Prasad EW. The effect of Ubretid on bladder function after recent complete spinal cord injury. *Br J Urol.* 1974 Apr;46(2):187–92.
- Philp NH, Thomas DG. The effect of distigmine bromide on voiding in male paraplegic patients with reflex micturition. *Br J Urol.* 1980 Dec;52(6):492–6.
- Bougas DA, Mitsogiannis IC, Mitropoulos DN, Kollaitis GC, Serafetinides EN, Giannopoulos AM. Clinical efficacy of distigmine bromide in the treatment of patients with underactive detrusor. *Int Urol Nephrol.* 2004;36(4):507–12.
- Skolarikos A, Tyritzis S, Stamatou K. Cholinergic drugs for treatment of recurrent urinary retention in high surgical risk/elderly BPH patients. A pilot study. *Minerva Urol Nefrol.* 2012 Sep;64(3):209–16.
- Frazier EP, Peters SL, Braverman AS, Ruggieri MR Sr, Michel MC. Signal transduction underlying the control of urinary bladder smooth muscle tone by muscarinic receptors and beta-adrenoceptors. *Naunyn Schmiedeberts Arch Pharmacol.* 2008 Jun;377(4-6):449–62.
- Brown C, Mans C. Urethral catheterization of the male guinea pig (*Cavia porcellus*). *Lab Anim (NY).* 2007 Jul-Aug;36(7):20–1.
- Horinouchi T, Aoki T, Akiyama R, Ono T, Shibano M, Tanaka Y, Koike K. Effects of distigmine, a long-acting cholinesterase inhibitor, on urinary bladder detrusor and urethra smooth muscles of guinea-pig: pharmacological analysis in vitro and in vivo. *Oyo Yakuri/Pharmacometrics.* 2003;64:45–52.
- Sekiya S, Ookawa N, Horinouchi T, Tanaka Y, Koike K. Effects of distigmine, a long-acting cholinesterase inhibitor, on urinary bladder contractile functions assessed by using cystometry method in anesthetized guinea-pigs. *Oyo Yakuri/Pharmacometrics.* 2006;70:29–34.
- Sekiya S, Michikawa H, Tanaka Y, Koike K. Effects of distigmine on the intraurethral pressure of anesthetized guinea-pigs. *Oyo Yakuri/Pharmacometrics.* 2006;71:19–27.

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K.O. and Y.T. designed and wrote the review. All authors accepted the final version of the review.

- 19 Sekiya S, Takahashi H, Seki Y, Teraoka A, Aikawa N, Tanaka Y, Koike K. Comparison of the effects of distigmine and neostigmine on guinea-pig urinary bladder contractile functions assessed by in vivo and in vitro studies. *Öyö Yakuri/Pharmacometrics*. 2008;75:85–96.
- 20 Obara K, Aikawa N, Sato K, Chino D, Tanaka Y. Comparison of the effects of distigmine and bethanechol on guinea-pig lower urinary tracts contractile functions assessed by in vivo and in vitro studies. *Öyö Yakuri/Pharmacometrics*. 2013;85:101–14.
- 21 Herzfeld E, Kraupp O, Pateisky K, Stumpf C. [Pharmacological and clinical effects of cholinesterase antagonist hexamethylene-bis(N-methylcarbaminoyl-1-methyl-3-oxypyridiniumbromide); (BC 51)]. *Wien Klin Wochenschr*. 1957 Apr;69(14):245–8.
- 22 Ito Y, Harada T, Fushimi K, Kagawa Y, Oka H, Nakazawa H, et al. Pharmacokinetic and pharmacodynamic analysis of acetylcholinesterase inhibition by distigmine bromide in rats. *Drug Metab Pharmacokinet*. 2010;25(3):254–61.
- 23 Breuel HP, Bohn-Olszewsky W, Engelsen SJ, Samhaber EM, Niklaus H. Inhibition of acetylcholinesterase by distigmine bromide (Ubretid). *Int J Clin Pharmacol Ther Toxicol*. 1993 May;31(5):230–5.
- 24 Obara K, Chino D, Tanaka Y. Distigmine bromide produces sustained potentiation of guinea-pig urinary bladder motility by inhibiting cholinesterase activity. *Biol Pharm Bull*. 2017;40(6):807–14.
- 25 Vree TB, Waitzinger J, Hammermaier A, Radhofer-Welte S. Absolute bioavailability, pharmacokinetics, renal and biliary clearance of distigmine after a single oral dose in comparison to i.v. administration of ¹⁴C-distigmine-bromide in healthy volunteers. *Int J Clin Pharmacol Ther*. 1999 Aug;37(8):393–403.
- 26 Obara K, Ogawa T, Chino D, Tanaka Y. The Long-Lasting Enhancing Effect of Distigmine on Acetylcholine-Induced Contraction of Guinea Pig Detrusor Smooth Muscle Correlates with Its Anticholinesterase Activity. *Biol Pharm Bull*. 2017;40(7):1092–100.
- 27 Kryger G, Silman I, Sussman JL. Structure of acetylcholinesterase complexed with E2020 (Aricept): implications for the design of new anti-Alzheimer drugs. *Structure*. 1999 Mar;7(3):297–307.
- 28 Obara K, Chino D, Tanaka Y. Long-lasting inhibitory effects of distigmine on recombinant human acetylcholinesterase activity. *Biol Pharm Bull*. 2017;40(10):1739–46.
- 29 Perola E, Cellai L, Lamba D, Filocamo L, Brufani M. Long chain analogs of physostigmine as potential drugs for Alzheimer's disease: new insights into the mechanism of action in the inhibition of acetylcholinesterase. *Biochim Biophys Acta*. 1997 Nov;1343(1):41–50.