Effects of Antimuscarinic Drugs on Both Urinary Frequency and Cognitive Impairment in Conscious, Nonrestrained Rats

Toshinori Oka*, Koushi Nakano, Tsukasa Kirimoto and Naosuke Matsuura

Pharmacology Research Laboratory, Taiho Pharmaceutical Co., Ltd., 224-2, Ebisuno, Hiraishi, Kawauchi-cho, Tokushima 771-0194, Japan

Received February 16, 2001 Accepted May 30, 2001

ABSTRACT—Recent studies indicate a risk of learning and memory impairments when patients with senile dementia are treated with antimuscarinic drugs. In this study, we compared the effectiveness of propiverine hydrochloride (propiverine) and oxybutynin chloride (oxybutynin) on the increased urinary frequency and cognitive impairment induced by nucleus basalis magnocellularis (nBM) lesioning in conscious and non-restrained rats. For examination of bladder function, nBM-lesioned rats were given total parenteral nutrition regimens for 8 days. Propiverine administered orally at 0.3, 3 and 30 mg/kg on the postoperative day 7 significantly lessened the increase in the frequency of voiding caused by the nBM lesion, whereas oxybutynin administration did not show any improvement at 0.1 or 1 mg/kg but did so at 10 mg/kg. To examine the memory impairment, we trained nBM-lesioned rats in an 8-arm radial maze task for 20 days and then evaluated the effectiveness of oral drug administration on 19th and 20th radial maze performance. The higher rate of errors caused by nBM lesioning was significantly aggravated by oxybutynin at 30 and 100 mg/kg. Propiverine showed slight aggravation of errors, but with no statistical significance at any dose, 30, 100 or 300 mg/kg. These results suggest that propiverine has comparatively less effect on the cognitive impairment than oxybutynin.

Keywords: Antimuscarinic, Propiverine, Oxybutynin, Dementia, Urinary frequency

Recently, the number of patients with abnormally high urinary frequency and/or urinary incontinence attributed to neurogenic or unstable bladder has been increasing. In particular, the number of patients with neurogenic bladder that results from functional disturbance of the brain (for example, dementia and cerebrovascular disease) is expected to increase concomitantly with the aging of society and the increase in the frequency of traffic accidents. As urinary bladder contraction is controlled largely by parasympathetic nerves, a peripherally active anticholinergic (antimuscarinic) drug, for example, oxybutynin or propiverine (1, 2), is generally used for the treatment of urinary frequency and incontinence. On the other hand, scopolamine, a centrally active anticholinergic agent, has been found to create transient memory impairments more frequently in the Alzheimer-type dementia patients than in age-matched elderly control subjects (3 – 5). Depending on the situation, antimuscarinic therapeutic drugs given for the treatment of increased urinary frequency and incontinence would also thus have the potential to create transient memory impairment in patients with senile dementia. However, there have been no clinical or animal studies reported to show whether these drugs affect the memory impairment in dementia or not.

Furthermore, under conscious and nonrestrained conditions, there have been few animal studies that have examined the effects of antimuscarinic therapeutic drugs on urinary frequency. These conditions are considered to be essential to assess the clinical efficacy of orally administered therapeutic drugs, because several subtypes of the muscarinic receptor in the central and peripheral nervous systems (including prejunctional and postjunctional) are involved in regulation of the urinary bladder (6). The usual methods using urinary bladder strips or cystometry under anesthesia or decerebration are impossible for the examination of the urinary bladder function mediated via the systemic antimuscarinic action.

By using the nucleus basalis magnocellularis (nBM)-lesioned rat as a cerebral dysfunction model, we developed a new urinary frequency model that provides frequency-volume charts obtained under the conscious and non-
restrained conditions. In our model, the loss of body weight is kept to a minimum by administration of total parenteral nutrition (TPN), thereby providing 100% survival with maintenance of an adequate and constant urine volume that enables precise evaluation of the effectiveness of the drugs tested. Using this model, we compared the effectiveness of propiverine and oxybutynin on reducing urinary frequency. In addition, another experiment was designed to investigate whether these drugs affected the memory impairment in nBM-lesioned rats that had been trained in an 8-arm radial maze task. Thus, we compared propiverine and oxybutynin in terms of their effect on both bladder function and memory impairment in this study.

**MATERIALS AND METHODS**

**Drugs**

Propiverine hydrochloride (1-methyl-4-piperidyl diphenyl-propoxyacetate hydrochloride) (Apogepha Arzneimittel GmbH, Dresden, Germany) and oxybutynin hydrochloride (Sigma, St. Louis, MO, USA) were dissolved in water. Scopolamine hydrobromide (Sigma) was dissolved in physiological saline.

**Rat model with bilateral lesioning of nBM**

Male Wistar rats (8-week-old) under sodium pentobarbital anesthesia (45 mg/kg intraperitoneally) were operated on according to the methods of Dubois et al. (7) and Hara et al. (8). The animals were placed in a stereotoxic apparatus and subjected to bilateral lesioning of the brain by ibotenic acid injection. The coordinates of the cannula placements used were the following: anterior, 7.7 mm; lateral, 2.4 mm; ventral, 7.1 mm according to Paxinos and Watson (9). Ibotenic acid (Sigma) was infused through a 0.4-mm diameter stainless steel needle connected via Teflon tubing to a microsyringe pump. The acid was dissolved in Dulbecco’s phosphate-buffered saline (D-PBS, pH 7.4) at a concentration of 10 μg/ml and infused in a volume of 0.75 μl for 3 min by means of the microsyringe pump. The sham-operated rats received only needle insertion into the nBM.

**TPN**

The rats were cannulated for parenteral infusion by the method described earlier (10), having been starved overnight prior to the bilateral lesioning of the nBM. A silastic catheter was then inserted into the right atrium of the heart via the right jugular vein, tunneled subcutaneously, and brought through the skin at the back of the neck. The catheter was protected at the exit point by being passed through a stainless steel spring sewn onto the skin. The catheter and spring were then connected to a flow-through swivel that permitted continuous infusion of the animal while it was in a metabolic unit under nonrestraint for 8 days. On the day of the operation, the infusion rate of TPN solution was 25.5 kcal/28 ml per day; and on day 1 and thereafter for an additional 7 days, the rats received complete parenteral nutrition that provided 51 kcal/56 ml per day. TPN solution was formulated under aseptic conditions by mixing 900 ml of Aminotripa2® (Otsuka Pharmaceutical Factory Co., Ltd., Tokushima), 0.67 ml of Solhvit® (Fuso Ltd., Osaka), and 0.2 ml of Elemexit® (Hoechst Marion Roussel Ltd., Tokyo). The rats infused with the TPN solution were housed in a controlled environment and exposed to a 12-h light / 12-h dark cycle (lights on 6:00 – 18:00).

**Frequency-volume chart**

Rats were randomly divided into the sham-operated group (n = 10) and nBM-lesioned group (n = 80) before the operation. On day 7 of TPN, the nBM-lesioned group was further divided into seven experimental groups, control (distilled water administered) group, propiverine (0.3, 3 and 30 mg/kg)-administered groups and oxybutynin (0.1, 1 and 10 mg/kg)-administered groups, to minimize the variance in urinary frequency that was observed by pre-analysis of the frequency-volume chart from 19:00 on day 6 to 4:00 on day 7. Fifteen rats in TPN groups were omitted from the analysis of results because of model failure (frequency of voiding was less than 20 in pre-analysis) or technical problems concerning catheters encountered during the infusion period. The final number of animals used in each group is indicated in each figure. On day 7 at 18:00, either drug or distilled water (in sham-operated and control groups) was orally administered at a volume of 5.0 ml/kg by use of a gastric needle. The monitoring of the frequency-volume chart was begun 1 h after the drug administration and lasted for 9 h. Voided urine was cumulatively collected into a urine cup containing liquid paraffin that was placed on a microbalance HF-200 (A&D Co., Ltd., Tokyo). The analog voltage from the microbalance was received by a MacLab8S (AD Instruments Pty Ltd., NSW, Australia) and processed by PowerLab/sv3.6 (proprietary client software); thereby, the identification limit of urine weight was 0.02 g. The frequency of voiding and the mean voided volume for 9 h was calculated by use of the proprietary client software.

**Radial 8-arm maze task**

Rats were randomly divided into the sham-operated group (n = 9) and nBM-lesioned group (n = 101) before the operation. Operated rats were provided a highly concentrated liquid diet (1.5 kcal/ml), Sanet® (Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya) and water ad libitum for 7 days. In the case of a marked decline in body weight, Sanet® was orally administered by use of a gastric needle.
On day 7 and thereafter for an experimental period, the rats were provided laboratory rat food pellets, the amount of which for each rat was adjusted daily so that their body weight would be maintained at about 75–85% of the free-feeding level. On day 8 and thereafter for an additional 4 days, each animal was allowed to adapt to the 8-arm radial maze. The 8-arm radial maze consisted of an octagonal platform (30 cm across) with eight arms (60 × 12 cm) radially extending from the platform. The maze was 50 cm above the floor. Small cups (3 cm in diameter and 1 cm in depth) were mounted at the end of each arm as receptacles for reinforcer (45 mg pellet; Bio-Serv, NJ, USA). Guillotine-type doors surrounded the platform and controlled access to each arm. Maze learning was started 14 days after the surgical operation by the methods described by Nanri et al. (11). Each rat was placed individually on the central platform with all guillotine doors closed, and then all of the doors were opened simultaneously to allow the animal free access to the arms. Entry into an arm that a rat had not previously visited was recorded as a correct choice, and re-entry was counted as an error. The number of errors and the number of initial correct choices (i.e., the number of correct responses before the first error) were used as indices of radial maze performance. The trial result was judged when the rat had visited all eight arms or had spent 10 min in the maze. Each rat was tested once daily for 20 days. Data were shown as the average of two trials, each consisting of 10 sessions. Surviving nBM-lesioned rats (n = 73) were further divided into eight groups, i.e., control (distilled water given orally) group, scopolamine (0.5 mg/kg given subcutaneously) group, propiverine (30, 100 and 300 mg/kg given orally) groups, oxybutynin (10, 30 and 100 mg/kg given orally) groups, to minimize variance of both initial correct and number of errors that was obtained with the trial on session 9. The final number of animals used is indicated in each figure. In session 10, either oral drug or distilled water (in sham-operated and control groups) was given to rats 1 h before the radial maze learning performance test. Scopolamine was given subcutaneously to rats 30 min before the test.

Statistics

Data obtained from the frequency-volume chart and maze learning performance studies were expressed as the mean ± S.E.M. and analyzed by the paired t-test and Wilcoxon/Kruskal-Wallis test, respectively. A probability value (P) of less than 0.05 was considered to be statistically significant.

RESULTS

Frequency-volume chart

Using a nBM-lesioned rat TPN model, we recorded the frequency-volume charts. In this model, none of the model rats died due to the nBM lesion during the experiments. Figure 1 shows the typical pattern of night micturition (19:00–4:00) in sham-operated and nBM-lesioned groups. The later group presented with hyperkinesia, and the body weight (211.7 ± 3.8 g) was less than that in the sham-operated group (233.4 ± 1.9 g) in spite of isovolemic and isocaloric complete parenteral nutrition. Furthermore, the total voided volume for 9 h in the nBM-lesioned group (8.89 ± 0.51 g) was also less than that of the sham-operated group (13.46 ± 0.68 g). In this experiment, therefore, the mean voided volume was indicated by the ratio of it to body weight.

Fig. 1. Typical patterns of night micturition (19:00–4:00). The sham-operated or nBM-lesioned rats received TPN solution for 8 days. On day 7–8, the frequency-volume charts were recorded for 9 h with consciousness and nonrestraint as described in the text. Each dotted line expresses a micturition. A: sham-operated rat and B: nBM-lesioned rat.
An increased frequency of voiding (Fig. 2) and decreased mean voided volume (Fig. 3) were observed in the control (nBM-lesioned) group as compared with the values for the sham-operated group. Propiverine administration at 0.3, 3 and 30 mg/kg significantly reduced the increase in urinary frequency caused by nBM lesioning and increased the voided volume when given at 0.3 and 30 mg/kg.

Whereas, oxybutynin only at its highest dose (10 mg/kg) significantly improved the values for urinary frequency and voided volume. Oxybutynin administration at 0.1 and 1 mg/kg did not result in any significant improvement. In addition, the highest dose of each drug, 30 mg/kg of propiverine or 10 mg/kg of oxybutynin, significantly increased the total voided volume for 9 h as compared with the values for the control group (30 mg/kg of propiverine: 58.6 ± 2.4, 10 mg/kg of oxybutynin: 60.0 ± 3.3, control group: 42.1 ± 2.5 g/kg B.W.).

Radial 8-arm maze task

In session 9 in the 8-arm radial maze task, neither the number of errors nor that of initial correct choices was different from the control group in any of the drug-treated groups (data not shown). In session 10, as shown in Fig. 4, the higher rate of errors caused by nBM lesioning was significantly aggravated by subcutaneous administration of scopolamine (a positive-control drug) at 0.5 mg/kg and by oral administration of oxybutynin at 30 and 100 mg/kg. However, oral administration of propiverine at 100 and 300 mg/kg showed only slight, nonsignificant, aggravation of errors. Furthermore, the lowering of the number of initial correct choices caused by nBM lesioning was significantly aggravated by subcutaneous administration of scopolamine or oral administration of oxybutynin at 100 mg/kg (Fig. 5). However, no aggravating effect on the initial correct choices was shown by propiverine administration.
**DISCUSSION**

We produced nBM lesions in rats by using ibotenic acid and compared the actions of propiverine and oxybutynin in these animals from the standpoint of both their effect on bladder function and adverse effects on the central nervous system. For strict comparison of the effective doses for both actions, it is necessary to use the same animal model and to compare their respective effective doses under the same conscious and nonrestrained conditions. The ibotenic acid-induced nBM lesion model that produces cholinergic nerve hypofunction in the cerebral cortex is well known as a model of impaired memory (12–14), and it is widely used as a model to confirm the efficacy, by serial administration, of anti-dementia drugs in the conscious state (15). In this study, we showed for the first time that this model makes it possible not only to observe the memory-ameliorating effect of anti-dementia drugs but also to evaluate memory disorder exacerbation by antimuscarinic drugs by using scopolamine as a positive-control drug. On the other hand, reports have also been published stating a significant reduction in bladder capacity in this model in cystometry experiments on conscious restrained rats (16). We established a method for analyzing frequency-volume charts with an adequate and constant urine volume by TPN. Using this model, we confirmed the clinical-like increased urinary frequency in the lesioned rats under conscious nonrestrained conditions.

We then compared the degree of action of propiverine and of oxybutynin on urinary frequency and cognitive impairment by administering either drug orally, which is the clinical route of administration.

The results showed that propiverine’s urinary frequency-ameliorating action was already expressed at the 0.3 mg/kg dose and that it almost perfectly reflected the clinical dose range in Japan (administration of 20–40 mg a day, approximately 0.33–0.67 mg/kg). In contrast, no effect of oxybutynin was observed at 0.1 mg/kg, the clinical dose of oxybutynin (administration of 6–9 mg a day, approximately 0.1–0.15 mg/kg); and this drug had no effect at all on urinary frequency even at 10 times that dose. A review of the literature (16) showed that oral administration of oxybutynin was reported to increase the bladder capacity of conscious restrained rats at 0.1 mg/kg, but that it had no effect on bladder capacity at 1/3 or 3 times that dose, suggesting that the effective dose range of oxybutynin in the rat is very narrow. Furthermore, the low efficacy of oxybutynin at 0.1 mg/kg might be due to it’s powerful antimuscarinic action (1), which results in decreased micturition pressure and increased residual urine volume.

In the experiment in which the radial maze was used, reference memory and working memory were necessary, and working memory has been claimed to be associated with the proper functioning of the cholinergic nervous system (17). The impairment of the acquisition trial score by scopolamine appeared to be due to impairment of working memory based on its antimuscarinic action (17, 18). A memory-imparing action of scopolamine has been confirmed in humans as well, and Alzheimer-type dementia patients have been reported to be more susceptible to impairment in cognitive tests by scopolamine than normal subjects (3–5). We also confirmed that the memory-imparing effect of scopolamine was more pronounced in rats with ibotenic acid-induced lesions in their basal ganglia than in sham-operated rats (data not shown). In the case of ibotenic acid-induced nBM lesions in rats, the cholinergic neural transmission system is organically damaged; e.g., a significant reduction in the acetylcholine level was found in the frontal cortex (13). Thus, it can be easily inferred that acquisition trial impairment will occur suddenly if the small residual acetylcholine transmission system is inhibited by antimuscarinic drugs. In this model system, the number of errors was significantly worsened by oxybutynin at 30 mg/kg, and the number of initial correct choices and number of errors, by the drug at 100 mg/kg. Comparison of the memory-imparing dose of oxybutynin, 30 mg/kg, with the clinical doses used in Japan (approximately 0.1–0.15 mg/kg) revealed a 200–300 fold disparity, but there was only a threefold disparity compared with the 10 mg/kg

![Image](image-url)
effective dose in the rat urinary frequency model. The binding affinity of oxybutynin for the muscarinic receptors of the guinea pig cerebral cortex has been reported to be 13.9 times that of its affinity for the receptors in the urinary bladder (cerebral cortex, $K_i = 4.12$ nM; bladder, $K_i = 57.1$ nM) (19), and expression of oxybutynin’s central antimuscarinic action is presumed to be sufficient. Based on this finding, the activity of the urinary frequency-ameliorating drug oxybutynin is significant even though the drug does not display a memory-impairing action in the urinary frequency-ameliorating dose range, and there was fear that it might induce memory impairment based on its antimuscarinic activity. In contrast, although propiverine tended to adversely affect the initial correct choices and the number of errors, there was no significant effect at the high dose of 300 mg/kg. This inactive dose of propiverine on memory impairment was 1000 times greater than the urinary frequency-ameliorating dose of 0.3 mg/kg. Moreover, the results did not show a memory-impairing action of propiverine at high doses of 450 – 900-fold compared with the clinical dose range (approximately 0.33 – 0.67 mg/kg), either. One possible explanation for this difference between the two drugs is that the binding affinity of propiverine for the muscarinic receptors of the guinea pig cerebral cortex ($K_i = 190$ nM) is approximately 1/46 that of oxybutynin (19). It was earlier certified by that there is no difference in intracerebral transfer between the two drugs. That is, one report (20) stated that the intracerebral concentration of oxybutynin was $0.009 \pm 0.002 \mu g/g$ tissue 1 h after oral administration of the drug at dose of 1 mg/kg, and another (21) indicated a similar concentration for propiverine ($0.011 \pm 0.005 \mu g/g$ tissue) given under the same conditions.

We compared the actions of propiverine and oxybutynin in terms of both their effect on bladder function and their central adverse effects in nBM-lesioned rats, and although the possibility of propiverine-induced memory impairment was not ruled out, the results suggested that propiverine exerts less effect on dementia symptoms than oxybutynin.

REFERENCES

1. Yono M, Yoshida M, Wada Y, Kikukawa H, Takahashi W, Inadome A, Seshita H, and Ueda S: Pharmacological effects of tolterodine on human isolated urinary bladder. Eur J Pharmacol 368, 223 – 230 (1999)
2. Madersbacher H, Halaska M, Voigl R, Aloussi S, and Hofner K: A placebo-controlled, multicentre study comparing the tolerability and efficacy of propiverine and oxybutynin in patients with urgency and urge incontinence. BJU Int 84, 646 – 651 (1999)
3. Sunderland T, Tariot PN, Cohen RM, Weingartner H, Mueller EA 3d, and Murphy DL: Anticholinergic sensitivity in patients with dementia of the Alzheimer type and age-matched controls.
4. A dose-response study. Arch Gen Psychiatry 44, 418 – 426 (1987)
5. Sunderland T, Tariot P, Murphy DL, Weingartner H, Mueller EA and Cohen RM: Scopolamine challenges in Alzheimer’s disease. Psychopharmacology (Berl) 87, 247 – 249 (1985)
6. Sunderland T, Tariot PN, and Newhouse PA: Differential responsivity of mood, behavior, and cognition to cholinergic agents in elderly neuropsychiatric populations. Brain Res 472, 371 – 389 (1988)
7. Somogyi GT and de Groot WC: Function, signal transduction mechanisms and plasticity of presynaptic muscarinic receptors in the urinary bladder. Life Sci 64, 411 – 418 (1999)
8. Dubois B, Mayo W, Agid Y, Le Moal M, and Simon H: Profound disturbances of spontaneous and learned behaviors following lesions of the nucleus basalis magnocellularis in the rat. Brain Res 338, 249 – 258 (1985)
9. Oka T, Ohwada K, Nagao M, and Kitazato K: Effect of arginine-enriched total parenteral nutrition on the host-tumor interaction in cancer-bearing rats. JPN J Parenter Enteral Nutr 17, 375 – 383 (1993)
10. Oka T, Ohwada K, Nagao M, and Kitazato K: Effect of arginine-enriched total parenteral nutrition on the host-tumor interaction in cancer-bearing rats. JPN J Parenter Enteral Nutr 17, 375 – 383 (1993)
11. Nanri M, Miyake H, Murakami Y, Matsumoto K, and Watanabe H: GTS-21, a nicotinic agonist, attenuates multiple infarctions and cognitive deficit caused by permanent occlusion of bilateral common carotid arteries in rats. Jpn J Pharmacol 78, 463 – 469 (1998)
12. Hepler DJ, Wenk GL, Cribs BS, Olton DS, and Coyle JT: Memory impairments following basal forebrain lesions. Brain Res 346, 8 – 14 (1985)
13. Kato H, Aikawa H, Yamamoto M, Shigeta S, and Shinohara Y: Effect of physostigmine on acetylcholine and monoamine metabolites in rat frontal cortex with lesions of the nucleus basalis of Meynert. Rinsho Shinkeigaku 34, 224 – 228 (1994)
14. Bednar I, Zhang X, Dastanj-Sedghi R, and Nordberg A: Differential changes of nicotinic receptors in the rat brain following ibotenic acid and 192-IgG saporin lesions of the nucleus basalis magnocellularis. Int J Dev Neurosci 16, 661 – 668 (1998)
15. Bhattacharya SK and Kumar A: Effect of Trasina, an ayurvedic herbal formulation, on experimental models of Alzheimer’s disease and central cholinergic markers in rats. J Altern Complement Med 3, 327 – 336 (1997)
16. Yamamoto T, Ikobuchi Y, Miura S, Sawada T, Ozaki R, Esumi K, and Ohitsuuka M: Effects of vamicamide on urinary bladder functions in conscious dog and rat models of urinary frequency. J Urol 154, 2174 – 2178 (1995)
17. Fader AJ, Johnson PE, and Dohanich GP: Estrogen improves working but not reference memory and prevents amnestic effects of scopolamine of a radial-arm maze. Pharmacol Biochem Behav 62, 711 – 717 (1999)
18. Fitzgerald RE, Berres M, and Schaeppi U: Validation of a radial maze test for assessing learning and memory in rats. Toxicology 49, 425 – 432 (1988)
19. Nagao M, Kaneko S, Hirota T, Isogai M, and Shimizu H: Effects of propiverine hydrochloride (propiverine) on the muscarinic
receptor binding affinity in guinea pig tissues and on salivation in conscious dogs. Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi) 113, 157–166 (1999) (text in Japanese with English abstract)

20 Akimoto Y, Kobayashi H, Shinozaki Y and Urakubo G: Studies on the metabolic fate of oxybutynin hydrochloride (1), Absorption, distribution and excretion in rats and dogs. Iyakuhin Kenkyu 15, 519–535 (1984)

21 Yamamoto Y, Tsuda M, Uda K, Shindo T and Kawaguchi Y: Pharmacokinetic studies of propiverine hydrochloride (1). Absorption, distribution and excretion after a single administration to rats. Xenobio Metabol and Dispos 4, 537–551 (1989)