JPP Journal of Pharmacy and Pharmacology

JPP 2009, 61: 1229–1232

Received December 25, 2008
Accepted June 9, 2009
DOI 10.1211/jpp/61.09.0013
ISSN 0022-3573

Research Paper

Synthesis of a quaternary bis derivative of imipramine as a novel compound with potential anti-enuretic effect

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Abstract

Objectives Imipramine has been used for over four decades (early reports in 1960s) for the treatment of nocturnal enuresis, although the reason for its effect is not clear. Imipramine is a tertiary amine, which may act both in the periphery and/or pass through the blood–brain barrier (BBB) in unionized form and exhibit a central effect. Since imipramine has anti-cholinergic properties, some believe it may exert its anti-enuretic effect by affecting peripheral cholinergic receptors, i.e. its anti-enuretic effect may be due to peripheral anti-cholinergic properties, whereas others think it can pass through the BBB and interact with central nervous system (CNS) receptors. If the anti-enuretic effect of imipramine is due to its peripheral anti-cholinergic effects, its entrance into the CNS is unnecessary. Therefore, the synthesis of a form of imipramine that can exhibit peripheral anti-cholinergic effects but does not have CNS adverse effects would have a safer drug profile in this case. On the other hand, if the anti-enuretic effect of imipramine is primarily due to its action on the CNS, a form of imipramine that cannot pass through the BBB has no effect on nocturnal enuresis treatment and thus may help to clarify the mechanism of action of imipramine in nocturnal enuresis treatment.

Methods This article describes the synthesis and evaluation of the anti-cholinergic effect of a new bis derivative of imipramine, which contains two imipramine units in its structure.

Key findings The compound exhibited anti-cholinergic activity comparable with that of imipramine on isolated guinea pig ileum.

Conclusions Being a quaternary ammonium, this compound is not expected to be able to cross the BBB and thus would cause fewer CNS side effects.

Keywords anti-cholinergic; anti-enuretic; imipramine; quaternary derivative

Introduction

Bedwetting or enuresis is a common symptom with many causes. According to the International Children's Continence Society (ICCS), nocturnal incontinence and enuresis are now synonymous. To add extra clarity, enuresis is sometimes called nocturnal enuresis. ICCS defined enuresis as children older than age five that have urine leakage in discrete amounts during the night while sleeping.[1] The aetiology of nocturnal enuresis remains unclear and a variety of factors may contribute to its development, such as genetic factors, stressful early-life events, sleep, physiological disturbances such as nocturnal polyuria, small functional bladder capacity and decreased arousal response to the full bladder and neurological and psychological influences.[2,3] The prevalence of this problem affects about 5 to 7 million children in the USA and occurs three times more often in boys than in girls.[4,5] It is now generally accepted that 15–20% of children will have some degree of nighttime wetting at seven years of age. In broad terms, in epidemiological surveys from various parts of the world that have looked at the prevalence of enuresis in different populations, the prevalence is 15–22% of boys and 7–15% of girls. The prevalance decreases with age, leaving approximately 1–3% by adulthood.[6] Unfortunately, only about one-third of the families of children with this frequently troubling problem seek help from a physician.[7]

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Most children will eventually outgrow their enuresis but this may take several years, and thus treatment is indicated for children who are adversely affected by the wetting. There are a number of therapeutic options that are widely used. These options are divided into pharmacological and non-pharmacological categories. Pharmacological treatments include imipramine (Tofranil, Novartis), oxybutynin, desmopressin (DDAVP)[2,6] and propiverine.[8] These drugs have been used with different degrees of success. In one randomized controlled trial, the efficacy of desmopressin versus imipramine was studied. The results showed no difference between the effectiveness of these drugs but the adverse effects of imipramine were more serious.[2] However, Chertin *et al.* showed that combination therapy with desmopressin and oxybutynin is more effective than imipramine.[9]

Tricyclic antidepressants, including imipramine, have been used extensively during the past 25 years in the treatment of nocturnal enuresis.[6,10] Imipramine provides some benefit in approximately 50% of children with nocturnal enuresis. The most important side effect of imipramine is cardiotoxicity at high doses, which occurs frequently. So imipramine should not be considered as a first-line treatment for nocturnal enuresis. If other treatments fail, imipramine can be used once daily, 1 h before bedtime.[4,6,10]

The mechanism of imipramine in enuresis treatment is not fully understood but it is thought to work via a number of effects, including an anti-cholinergic effect and effects on arousal, the sphincter and urine production.[4,6,10] According to one theory, the bladder detrusor muscle of various species (including humans) contains a variety of muscarinic receptor subtypes in which M_2 and M_3 receptors are predominant, with the M_2 subtype outnumbering the M_3 receptor subtype (3 : 1 ratio).[11] The peripheral anti-cholinergic effect of imipramine may decrease the bladder contractility and lead to increased bladder filling and improved voided volumes.[4,5] On the other hand, imipramine can pass through the blood–brain barrier (BBB) and act there as an anti-cholinergic drug. All muscarinic receptor subtypes (M_1–M_5) are present in various regions of the human brain. These receptors in the brain activate a multitude of signalling pathways, which are important for the feedback regulation of acetylcholine release, modulation of neuronal excitability and synaptic plasticity.[11,12] Studies linking enuresis to rapid eye movement sleep have suggested that imipramine may exert its anti-enuretic effect through its action on the central nervous system (CNS).[5,10]

If the anti-enuretic effect of imipramine is related to its peripheral anti-cholinergic effects, it is not necessary for it to pass through the BBB to the CNS, where it could potentially have adverse effects. Thus, synthesis of a form of imipramine that exhibits peripheral anti-cholinergic effects and does not have CNS adverse effects has the potential for great clinical utility. On the other hand, if the anti-enuretic effect of imipramine is due to its action on the CNS, a form of imipramine that cannot pass through the BBB would be ineffective for nocturnal enuresis treatment. Therefore, this form of imipramine may help to clarify the mechanism of action of imipramine in nocturnal enuresis treatment. This idea prompted us to design and synthesize a bis derivative of imipramine containing a quaternary amine that has little likelihood of a CNS effect.

Materials and Methods

Synthetic method and spectroscopic details

All chemicals and reagents were obtained from Merck (Darmstadt, Germany) and were used without further purification. Melting points (mp) were determined using an Electrothermal 9100 apparatus. Infrared spectra were measured on a Perkin-Elmer 830 spectrometer using KBr pellets. ^{1}H-NMR spectra were recorded on a 400 MHz Bruker spectrometer. Mass spectra were recorded on an Agilent ion trap MSD VL equipped with an electro-spray ion source. Thin-layer chromatography (TLC) was performed on E. Merck silica gel 60-F-254 plates.

Bischlorodimethyl ether was synthesized according to the previously reported method.[13]

Imipramine base

To a solution of 6 g imipramine HCl in 90 ml water, a sufficient amount of 20% w/v sodium hydroxide solution was added to adjust the pH to 14. The resulting solution was then transferred to a separating funnel and was extracted three times with 40 ml chloroform each time. The chloroform layer, after being dried over anhydrous sodium sulfate, was evaporated to give 3.96 g of imipramine base as a white powder (66% yield); m.p. 99–102°C (dec.).

1,1′-[Oxybis(methylene)]bis-[10,11-dihydro-N, N-dimethyl-5H-dibenz[b,f]azepine-5-propanammonium dichloride

To a solution of 1.47 g (5 mmol) imipramine base in 10 ml of acetone, 0.287 g (2.5 mmol) bischolorodimethyl ether in 5 ml acetone was added. The reaction mixture was refluxed for 2 h and then was stirred at room temperature for 12 h. The precipitate was filtered and was rinsed with cold acetone. The solid was then crystallized from a mixture of absolute ethanol and ethyl acetate to give 0.95 g of white solid (54% yield). m.p. 125–134°C, IR (KBr, cm^{-1}): 3400, 2920, 1600, 1480, 1240, 1180, 1160, 1130, 1110.

^{1}H-NMR (DMSO – d_6): δ 7.13 (12H; m; 2,2′-H, 3,3′-H, 4,4′-H, 7,7′-H, 8,8′-H, 9,9′-H), 6.92 (4H; m; 1,1′-H, 10,10′-H), 5.20 (4H; S; 14,14′-CH_2), 3.77 (4H; t, J = 5.6 Hz; 13,13′-CH_2), 3.35 (4H; broad m; 11,11′-CH_2), 3.10 (8H; S; 5,5′-CH_2, 6,6′-CH_2), 2.92 (12H; S; CH_3), 1.90 (4H; broad t; 12,12′-CH_2).

ESI-MS: 302 (m^{2+}/2).

Biological evaluation

Male albino guinea pigs with 300–450 g body weight were used. They were fasted for 24 h and fed with water as required.[14,15] The animals were euthanized by stunning, incision into the carotid and exsanguination. They felt no unnecessary pain and the study protocol was approved by the ethics committee of the Shaheed Beheshti University of Medical Sciences (5907–83/9/17). Terminal ileum was

immediately dissected and placed in a beaker containing oxygenated Tyrode solution (137 mM NaCl, 207 mM KCl, 1.1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 0.4 mM NaH_2PO_4, 12 mM $NaHCO_3$ and 5 mM glucose). Each segment of the gut was placed in an organ bath, fixed by one side to a hook and by the other side to the isotonic myograph transducer (NBS 222–0152F). The preparation was maintained at 37°C and continuously bubbled with oxygen gas. An initial tension of 0.5 g was applied to the tissue, and a 60 min equilibrium period was allowed, washing every 15 min with fresh Tyrode solution. All responses were recorded on a physiograph (NBS MK-III-S). Dose–response curves for acetylcholine in the presence and absence of imipramine and its bis derivative were constructed. After each test, the tissue was washed and allowed to rest for approximately 10 min.[14,15]

The results are expressed as mean ± SEM and each point represents nine replicated samples. For comparison of dose–response curves, two-way ANOVA and a posthoc Tukey HSD (honestly significant difference) test were used. Based on the structural features of the bis derivative we assume it is a competitive reversible antagonist. Thus the pA_2 was calculated using Schild analysis and was used to compare the antagonistic strength of imipramine and its bis derivative (JMP software, Ver 5.1, SAS Institute Inc.).

Results and Discussion

Imipramine is a dibenzazepine derivative with an *N,N*-diethylaminopropyl side chain. Imipramine exists mostly in its ionized form at physiological pH (7.4). The high lipid solubility of the uncharged form of this drug, however, causes it to penetrate the BBB to a great extent.

One way to prevent imipramine from entering the CNS is by making the compound permanently charged, and this could be achieved by quaternization of its tertiary amine side chain. In order to make the minimum modification in the imipramine structure, a bis derivative of imipramine was designed and synthesized by the reaction of imipramine base with dichlorodimethyl ether in acetone as solvent (Figure 1).

The chemical structure of the newly synthesized derivative, which contains two quaternary imipramine units in its molecule, was confirmed by infrared, ^{1}H-NMR and electrospray-mass spectrometry. Anti-cholinergic activity of the compound was measured on the isolated ileum of the rat. The dose–response curve was constructed for acetylcholine and the ED50 was calculated for imipramine and its bis derivative (compound A) as the concentration which could cause a 50% decrease in the contraction of the isolated ileum. The results for imipramine and compound A are presented in Table 1. As shown in Table 1, a significant inhibition of acetylcholine-induced contraction has been caused by both imipramine and compound A. Dose–response curves for acetylcholine in the presence and absence of imipramine and its bis derivative are shown in Figures 2 and 3. According to Schild analysis, the pA_2 values for imipramine and its bis derivative are 7.06 (6.62–7.76) and 7.23 (6.79 ~ 7.99) respectively. The results indicate that imipramine and its bis derivative are equipotent and no statistically significant difference between the pA_2 values was observed.

The molecular weight of compound A is approximately twice the molecular weight of imipramine HCl. The molar equipotency of these two compounds could indicate that compound A interacts with cholinergic receptors through one of the imipramine units in its structure and the other imipramine unit does not interact simultaneously. This could be due to the fact that the two imipramine units are too close to each other. Further studies with longer linking groups between the two imipramine units could be helpful for clarification of this hypothesis. Since compound A has anti-cholinergic activity comparable to imipramine, it should have similar indications, but since it is also permanently charged, it will fail to penetrate the BBB. This would be a disadvantage if the anti-enuretic effect is caused by action of the drug on the CNS, but would be a great advantage if it is due to a peripheral anti-cholinergic action, since compound A would have the same therapeutic

Table 1 ED50 of acetylcholine in the presence of imipramine and its dimer at different concentrations

Experimental condition		ED_{50} (M)	Confidence interval 95% (M) (lower~upper)
Imipramine	Control	7.42×10^{-8}	$2.88 \times 10^{-8} \sim 1.89 \times 10^{-7}$
	1×10^{-7}	1.83×10^{-7}	$6.53 \times 10^{-8} \sim 4.65 \times 10^{-7}$
	1×10^{-6}	2.17×10^{-5}	$8.59 \times 10^{-6} \sim 5.72 \times 10^{-5}$
	1×10^{-5}	5.56×10^{-3}	$1.26 \times 10^{-3} \sim 2.86 \times 10^{-2}$
Imipramine dimer	Control	1.41×10^{-7}	$2.20 \times 10^{-8} \sim 7.87 \times 10^{-7}$
	1×10^{-7}	6.92×10^{-7}	$1.38 \times 10^{-7} \sim 3.30 \times 10^{-6}$
	1×10^{-6}	1.07×10^{-5}	$2.20 \times 10^{-6} \sim 5.24 \times 10^{-5}$
	1×10^{-5}	1.44×10^{-3}	$2.01 \times 10^{-4} \sim 1.65 \times 10^{-2}$

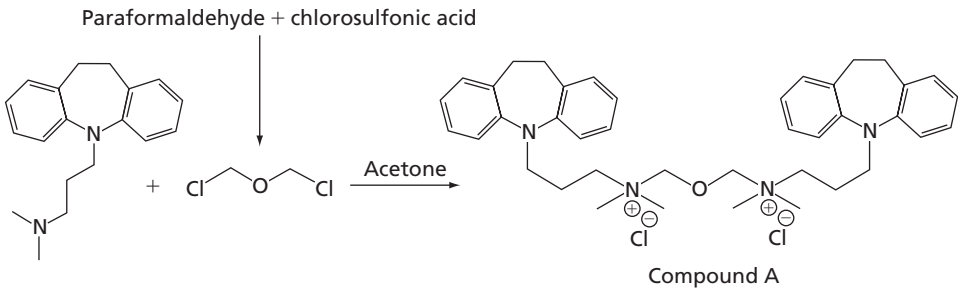

Figure 1 Preparation of imipramine dimer by the reaction of imipramine base with dichlorodimethyl ether in acetone as solvent

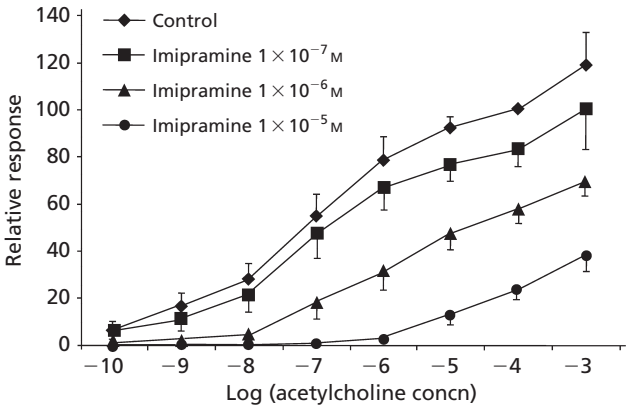

Figure 2 Dose–response curves of acetylcholine in the presence of imipramine. Dose–response curves are shown for different molar concentrations of imipramine in the isolated ileum of the guinea pig. Each point represents 9 replicate samples and the results are expressed as mean ± SEM.

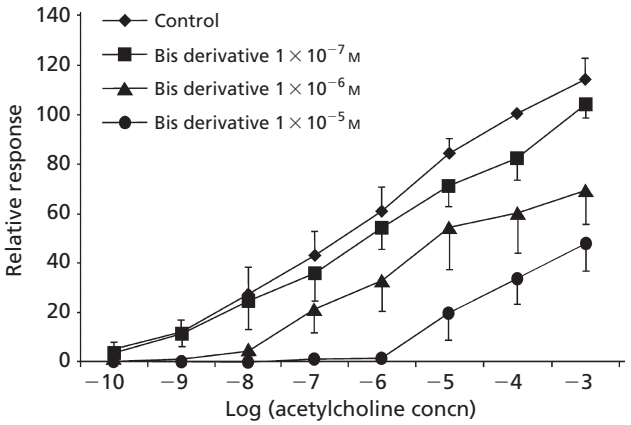

Figure 3 Dose-response curves of acetylcholine in the presence of imipramine bis derivative. Dose–response curves are shown for different molar concentrations of the imipramine bis derivative in the isolated ileum of the guinea pig. Each point represents 9 replicate samples and the results are expressed as mean ± SEM.

effect without exposing the patient to the CNS effects of imipramine.

One of the main disadvantages for imipramine is its cardiotoxicity. Since this side effect is due to the anti-cholinergic activity of this drug, the same disadvantage is anticipated for its bis derivative.

Conclusions

Our data confirm our assumptions that the imipramine bis derivative can block the spasmogenic effect of acetylcholine in a concentration-dependent manner. A parallel shift in the dose–response curves for both imipramine and its bis derivative suggests that both compounds are competitive antagonists of acetylcholine.

Guinea pig ileum preparations respond to most of the common spasmogens and are particularly suitable for the study of cholinergic receptors. However, isolated guinea pig detrusor muscle would be another suitable model for further in-vitro evaluation of the anti-cholinergic activity of compound A. On the other hand, measurement of intravesical bladder pressure in the intact animal could also be considered as a means to prove the anti-enuretic activity of this compound.

Declarations

Conflict of interest

The author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

The authors acknowledge the financial support of the Shaheed Beheshti Pharmaceutical Sciences Research Center for conducting the present study.

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