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TOLTERODINE - A NEW BLADDER SELECTIVE MUSCARINIC RECEPTOR ANTAGONIST: PRECLINICAL PHARMACOLOGICAL AND CLINICAL DATA

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Summary

Tolterodine is a new, potent and competitive muscarinic receptor antagonist in clinical development for the treatment of urge incontinence and other symptoms of unstable bladder. Tolterodine has a high affinity and specificity for muscarinic receptors *in vitro* and it exhibits a selectivity for the urinary bladder over salivary glands *in vivo*. A major active metabolite, (PNU-200577) the 5-hydroxymethyl derivative of tolterodine, has a similar pharmacological profile. Based on pharmacological and pharmacokinetic data, it has been concluded that this metabolite contributes significantly to the therapeutic effect of tolterodine. The bladder selectivity demonstrated by tolterodine and PNU-200577 *in vivo* cannot be attributed to selectivity for a single muscarinic receptor subtype. Moreover, this favourable tissue-selectivity seems to occur also in humans. Tolterodine is well tolerated and it exerts a marked effect on bladder function in healthy volunteers. Phase II data indicate that tolterodine is an efficacious and safe treatment for patients with idiopathic detrusor instability or detrusor hyperreflexia. An optimal efficacy/side-effect profile is obtained with tolterodine, at a dosage of 1 or 2 mg twice daily, which seems to have less propensity to cause dry mouth than the currently available antimuscarinic drugs.

Key Words: **urinary bladder, incontinence, dry mouth, *in vivo*, human**

Urinary incontinence is reported by 5-10% of the adult population and the prevalence, particularly of urge incontinence, increases with age (1). The symptoms of an unstable bladder comprise urge incontinence, urgency and frequency. Unstable bladder is presumably caused by uncontrolled detrusor contractions during the filling phase. It is generally agreed that contractions of the human bladder are mediated mainly by cholinergic muscarinic receptors (2). The bladders of various species, including humans, contain a mixed population of muscarinic M_2/m2 and M_3/m3 receptors (3). The M_2/m2 receptors predominate, but it is generally believed that the contractile response is mediated only by the M_3/m3 receptors (3). The pharmacological treatment of unstable bladder has for many years been based on muscarinic receptor antagonists (2) and oxybutynin is currently the drug of choice. The effectiveness of oxybutynin has been documented in several controlled clinical studies, but its usefulness is limited by classical antimuscarinic side-effects, which often leads to discontinuation of treatment (4). Dry mouth, for example, is experienced by at least 50% of patients treated with oxybutynin (4).

Tolterodine (PNU-200583, (R)-N,N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropan-amine) is a new muscarinic receptor antagonist under clinical development for the treatment of unstable bladder. In this paper, the antimuscarinic *in vitro* and *in vivo* profiles of tolterodine and a major active metabolite (the 5-hydroxymethyl derivative, PNU-200577, Labcode DD 01) are reviewed in comparison to those of oxybutynin and some other selective muscarinic receptor antagonists. Clinical data on tolterodine from phase I studies in healthy volunteers and from phase II studies in patients with detrusor hyperreflexia and idiopathic detrusor instability are also presented.

Preclinical pharmacology

Tolterodine is a potent and competitive inhibitor of carbachol-induced contractions of isolated urinary bladder preparations from guinea pig (K_B 3.0 nM) (5) and humans (K_B 4.0 nM) (6) and it binds with high affinity to muscarinic receptors in the bladder (Table I) and other tissues (5). Tolterodine does not exhibit any selectivity with respect to the human muscarinic receptors, expressed in Chinese hamster ovary (CHO) cells. The Ki values determined at m1-m5 receptors are (nM): 3.0, 3.8, 3.4, 5.0 and 3.4, respectively (7) (c.f. Table I). The *in vitro* profile of the 5-hydroxymethyl metabolite of tolterodine (PNU-200577), is similar to that of tolterodine (8).

Although M_2/m2 receptors predominate in the urinary bladder of various species, bladder contraction is considered to be mediated by the minor population of M_3/m3 receptors (3). This hypothesis is supported by functional *in vitro* data determined in the guinea pig urinary bladder (K_B, Table I) (7) for the selective reference compounds darifenacin (M_3 selective) (9), UH-AH 37 (low affinity for M_2/m2) (10) and AQ-RA 741 (M_2/m2 selective) (11). On the other hand, the data on oxybutynin do not fit into this scheme. Oxybutynin shows a distinct selectivity profile with respect to the human m1-m5 receceptors expressed in CHO-cells, with the highest affinity at m3 receptors (Ki 0.67 nM) > m4, m1 (Ki 2.0 and 2.4 nM) ≥ m2 (Ki 6.7 nM) ≥ m5 (Ki 11 nM) (7), but the K_B-value derived from studies on bladder contraction (4.4 nM) obviously correlates with binding data at m2, rather than at m3 receptors (Table I).

Table I

Functional (K_B) and Binding (Ki) Data in Guinea Pig Tissues and CHO-Cells

Drug	K_B (nM)	Ki (nM)			
	Urinary Bladder	Urinary Bladder	Parotid Gland	m2	m3
Tolterodine	3.0	2.7	4.8	3.8	3.4
PNU-200577	0.84	2.9	5.2	2.0	2.5
Oxybutynin	4.4	4.0	0.62	6.7	0.67
Atropine	0.70	1.6	0.85	2.9	1.0
Darifenacin	0.87	78	1.7	56	1.2
UH-AH 37	5.2	82	26	49	7.2
AQ-RA 741	140	12	170	4.4	55

Data from refs. 5, 7- 8.

Tolterodine and PNU-200577 have about 8 times lower affinity than oxybutynin in the parotid gland (M_3) and 4-5 times lower affinity at m3 receptors in CHO-cells (Table I) but they are not less potent than oxybutynin in inhibiting carbachol-induced contractions of the guinea pig bladder (5) (Table I). PNU-200577 is in fact 5 times more potent than oxybutynin in this respect. This cannot be explained by additional action(s) at other cellular targets, since both tolterodine and PNU-200577 show a very high degree of specificity for muscarinic receptors (5, 8 and Nilvebrant, unpublished data). Morover, both tolterodine and oxybutynin effectively inhibit electrically induced contractions of human detrusor strips, with IC50 values (tolterodine: 2.5 nM, oxybutynin: 3.2 nM) that are similar to the KB- and Ki- values determined in guinea pig bladder (12). The lack of correlation between functional data on bladder contraction and binding affinity at M_3/m3 receptors is not unique for oxybutynin. A similar profile has been found for dicyclomine: the KB value determined for dicyclomine in guinea pig bladder is 24 nM, (Nilvebrant, unpublished data), while the Ki -values determined in heart (M_2) and parotid gland (M_3) are 24 nM and 2.8 nM, respectively (13). Whether these data indicate that not only M_3/m3 receptors are involved in bladder contraction (14, 15) or that M_3 receptors may be heterogeneous (16), remains to be clarified.

Table II

Selectivity Profiles *In Vivo* and in *In Vitro* Binding Studies

Drug	Anaesthetised cat ID50 nmol/kg			Affinity ratios *in vitro*	
	Bladder Contraction	Salivation	Selectivity	Guinea pig Tissues Bladder/Parotid gland	CHOcells m2/m3
Tolterodine	101	257	Bladder	0.6	1.1
PNU-200577	15	40	Bladder	0.6	0.8
Oxybutynin	200	104	Salivary gland	6.5	10
Atropine	18	21	Non-selective	1.9	2.9
Darifenacin	119	99	Salivary gland	46	47
UH-AH 37	311	120	Salivary gland	3.2	6.8
AQ-RA 741	1060	1536	Bladder	0.07	0.08

Data from refs. (5, 7, 8, 17). Bladder contraction *in vivo* was induced by acetylcholine (i.a.) and salivation was induced by electrical stimulation of the chorda-lingual nerve. Antagonists were administered by intravenous infusion. Affinity ratios were calculated from the Ki-values displayed in Table I.

The most interesting feature of tolterodine and PNU-200577 is that they are significantly more potent in inhibiting urinary bladder contractions than salivation in the anaesthetised cat (17, 8) (Table II). This favourable selectivity profile can obviously not be attributed to selectivity for a single muscarinic receptor subtype. However, the reference compounds which in binding studies show a selectivity for parotid gland over bladder and for m3 over m2 receptors in CHO-cells, oxybutynin, darifenacin and UH-AH 37, are more effective in inhibiting salivation than bladder contraction *in vivo* (Table II). Thus, a selectivity for M_3/m3 over M_2/m2 receptors is not necessary for an effective inhibition of bladder contraction *in vivo*, but may result in a more pronounced effect on salivation (Fig. 1).

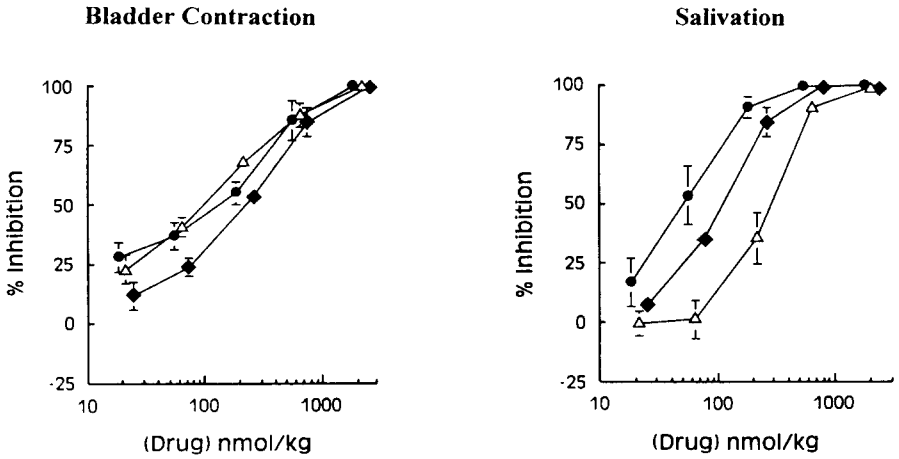

Fig. 1
Effects on urinary bladder contraction and salivation in the anaesthetised cat The rank order of potency for tolterodine (△), oxybutynin (◆) and darifenacin (●) is different with respect to these two responses. Plots constructed from data in (7).

The mechanism behind the bladder selectivity demonstrated for tolterodine and PNU-200577 *in vivo* is not known. Based on the combined *in vitro* and *in vivo* data, it may be speculated either that the M_3/m3 receptors in glands are more sensitive than those in the bladder, or that the M_3 receptors are heterogeneous (15). On the other hand, the M_2/m2 selective antagonist AQ-RA 741 exhibits a bladder selectivity which, although less pronounced, is similar to that demonstrated for tolterodine and PNU-200577 (7). This may suggest that M_2/m2 receptors are involved in bladder contraction (c.f. 14-16) and that blockade of M_2/m2 receptors in the bladder contributes to the favourable selectivity profiles of tolterodine and PNU-200577. However, muscarinic receptors involved in transmitter release are present on both adrenergic and cholinergic nerves in the detrusor of different species (2). A mixed prejunctional population of inhibitory M_2 and facilitating M_1 receptors has been demonstrated on postganglionic cholinergic nerves in the detrusor (18). The regulation of bladder function is complex and the relative functional importance of the different muscarinic receptors *in vivo* remains to be clarified. Nevertheless, the bladder selectivity demonstrated for tolterodine and PNU-200577 in the preclininical pharmacological *in vivo* studies seems to occur also in humans, as indicated by the data from the clinical phase I and II studies presented below.

Clinical pharmacokinetics/pharmacology

Tolterodine is rapidly absorbed and an approximate dose-proportional increase in peak serum levels is observed after about 1 h (19). Basic pharmacokinetic parameters are: half-life 2-3 h, systemic clearance about 30 l/h and volume of distribution about 110 l. The major metabolic pathway of tolterodine involves hydroxylation of the 5-methyl group, mediated by cytochrome P450 2D6, resulting in the 5-hydroxymethyl derivative PNU-200577 (19-21), which is

pharmacologically active (8). Similar mean peak serum concentrations (C_{max}) of tolterodine and PNU-200577 are found in most human subjects (20). The 5-hydroxymethyl metabolite is further metabolised to the corresponding carboxylic acid. The other primary metabolic pathway, which is of minor quantitative importance, involves N-dealkylation of tolterodine (21). Excretion of the 5-carboxylic acid and the N-dealkylated 5-carboxylic acid metabolites accounts for about 80% of the dose excreted in urine. The excretion of intact tolterodine is low (<1%) (19).

In the first clinical phase I study, tolterodine was given as single oral doses (0.2-12.8 mg) to healthy volunteeers (19). After 12.8 mg, heart rate was increased by 35% after 1 h and salivary secretion (induced by chewing of paraffin wax) was decreased. Long-lasting (until the next day) and marked effects on bladder function, such as micturition difficulties, were reported by the healthy volunteers (22). Effects on the bladder were noticed also when dry mouth was no longer reported. This was the first indication that the selectivity for bladder over salivary glands is present also in humans. In a later study (23), in which effects on bladder function were measured by cystometry, it was confirmed that tolterodine exerts a significant inhibitory effect on bladder function after a single oral dose of 6.4 mg. Stimulated salivary secretion was also inhibited, but only around C_{max}, while the effects on the bladder were more persistent. Thus, significant effects on cystometric variables were registered 5 h after dose and one subject experienced micturition difficulties up to 10 h after dose. No significant effects on blood pressure or heart rate were found. However, a considerable volume of residual urine was observed (23).

One subject was found to have significantly higher serum levels of tolterodine than the other volunteers, without showing divergent urodynamic effects. It was speculated that this subject was a poor metaboliser of tolterodine (23). Later, in the clinical phase II studies, the majority of patients in the four dose groups (0.5, 1, 2 and 4 mg) were found to have median peak serum concentrations of tolterodine of 0.4, 0.7, 1.5 and 3.8 µg/l, respectively, and concentrations of the 5-hydroxymethyl metabolite PNU-200577 in the same range (20). However, a small proportion of the patients showed a pharmacokinetic profile in accordance with poor metabolisers, having about ten times higher tolterodine concentrations but no measurable concentrations of the 5-hydroxymethyl metabolite. The unbound fraction of the metabolite in human serum is, however, 10-fold higher than for tolterodine (3.7%) (Påhlman and Gozzi, manuscript in preparation) and, together with the data on the antimuscarinic potency *in vitro* (8), this indicates that the 5-hydroxymethyl metabolite (PNU-200577) accounts for the major part of the pharmacological effect of tolterodine in extensive metabolisers. In Caucasians, it can be expected that about 7% of the population are poor metabolisers.

The conclusion from the early clinical phase I studies was that tolterodine shows a good tolerability over a wide dose-range. There was, however, an increase in urinary residual volumes after a dose of 6.4 mg and, therefore, 4 mg was selected as the highest dose for the clinical phase II studies. Despite the relatively short half-lives of tolterodine and PNU-200577, a twice daily dosage regimen was chosen because of the long-lasting effects on bladder function.

Clinical phase II studies

The safety, tolerability and efficacy of tolterodine have been evaluated in four randomised, double-blind, placebo controlled, parallel group, dose-ranging, multi-centre phase II studies. Patients with idiopathic detrusor instability (24) were recruited to two of these studies, while the

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