The Role of Sex, Age and Genetic Polymorphisms of CYP Enzymes on the Pharmacokinetics of Anticholinergic Drugs

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Abstract

Drugs exhibiting anticholinergic properties are commonly used by older adults even with the associated risk of adverse drug events. Aging, sex and genetic polymorphisms of cytochrome P450 (CYP) enzymes are associated with alterations in pharmacokinetic processes which may increase drug exposure and further increase the risk of adverse drug events. Age-related changes include; pseudocapillarization of liver sinusoidal endothelial cells which limit passage of drugs through the liver, an approximate 3.5% decline in CYP450 content for each decade of life, and a reduction in kidney function reducing drug excretion. Sex-related differences include; women having delayed gastric and colonic emptying, higher gastric pH, reduced catechol-O-methyl transferase activity, reduced glucuronidation, and reduced renal clearance and men having larger stomachs which may allow them to dissolve and absorb medication more completely. The overlay of poor metabolism phenotypes for CYP2D6 and CYP2C19 may further modify anticholinergic drug exposure in a significant proportion of the population. These factors help explain clinical trials that show older adults and specifically women achieve higher plasma concentrations of anticholinergic drugs. Despite this knowledge, age and sex are rarely considered when making decisions about the dosing of anticholinergic medications. As this is relevant to the future use of personalized medicine, the objective of this review is to provide a clinical perspective on age, sex, and CYP genetic polymorphisms and their role in the metabolism and exposure to anticholinergic drugs. Future work needs to account for age, sex and CYP polymorphism so that we may better approach personalized medicine for optimal outcomes.

Introduction

Anticholinergic medications are potentially inappropriate for older adults[1,2]. Clinical experience and research demonstrate an increased risk of serious adverse drug events and mortality related to the use of anticholinergic drugs in older adults (table 1). Due to variability in the anticholinergic activity of individual medications, one in isolation may fail to cause any noticeable effect but when two or three anticholinergic agents are combined the total anticholinergic burden can result in adverse events[3–10]. Anticholinergic medications may cause mild adverse effects such as; flushing, mydriasis with loss of accommodation, fever, constipation, urinary retention[11], or more serious effects such as cardiovascular events[10], delirium or cognitive impairment[5,12,13]. These adverse effects can result in emergency department visits[14], hospital admission[7] or death[10]. As older adults are at increased risk of these sequelae, the pharmacokinetics, pharmacodynamics, expected effect and toxicity are important to understand in this population.

Cytochrome P450 (CYP) enzymes mediate the metabolism and pharmacologic activity of anticholinergic medications. Age, sex and genetics can cause variation in CYP enzyme activity which may increase exposure to anticholinergic drugs and influence their clinical effect or toxicity[15]. The purpose of this review is to analyze the current knowledge on how age, sex, and genetic polymorphisms of CYP2D6, CYP2C19 and CYP3A4 affect the pharmacokinetics (absorption, distribution, metabolism, excretion) and subsequent pharmacologic response of anticholinergic drug, to equip clinicians with an understanding to better predict and avoid anticholinergic adverse events in older adults.

Methods

Data Sources for Review

The PubMed database was searched using all dates available (1950-January 2020) with the initial search terms age, sex, anticholinergic agent and pharmacokinetics. Results of the preliminary search lacked recent studies including human subjects (see appendix 1). A second search included limits of human subjects, English language and clinical trials. In this directed search each term; sex, age and CYP2C19, CYP2D6, CYP3A4 (the most common CYP enzymes involved in the metabolism of anticholinergic drugs), were searched in combination with anticholinergic and pharmacokinetics. This second search identified more specific and more recent studies. Anticholinergic drugs were considered any drugs appearing on the anticholinergic cognitive burden scale[16]. This review was not meant to be an exhaustive summary of all available literature on the topic but instead a review of the literature to inform clinical decision making about anticholinergic drug use in older adults. If insufficient studies were identified on a topic, further search was completed using the specific pharmacokinetic parameter of interest with each of the search terms sex, age and CYP2C19, CYP2D6 and CYP3A4. The Web of Science database was consulted to find citing articles. Further articles were taken from review articles examined during the literature searches.

Results

Anticholinergic Receptors and Signaling

The term anticholinergic agent refers to drugs that antagonize the muscarinic acetylcholine (M) receptor. The M receptor is a G-protein coupled receptor (GPCR) that resides on the cell membrane. It is comprised of 7 alpha helices that span the cell membrane and possesses an extracellular binding domain. When activated, the GPCR undergoes conformational change that induces dissociation of the trimeric G protein-complex into the free and active Gα and Gβγ subunits. The Gα and Gβγ subunits activate enzyme effectors or ion channels which regulate intracellular concentrations of secondary messengers such as cAMP, cGMP, diacylglycerol, IP3, DAG, arachidonic acid, sodium, potassium or calcium cations depending on the receptor subtype[17]. Gα and Gβγ activity is terminated by activation of an endogenous high-affinity GTPase located in the Gα subunit which hydrolyzes the terminal γ-phosphate of Gα-GTP to Gα-GDP which then binds Gβγ to reform the trimeric G protein- complex[18,19]. In response to prolonged signaling, receptors can be internalized by separation from the effector and binding to small endosomes. This desensitizes the receptor by reducing the number of receptors on the cell surface. This occurs in response to receptor phosphorylation which is often related to a hormone response[19,20]. The five M receptor subtypes and their associated functional response to agonism and antagonism are described in figure 1. M1, M3 and M5 receptors all couple with Gq/11 and lead to release of calcium from the sarcoplasmic reticulum. M2 and M4 receptors are coupled to Gi proteins and their activation leads to inhibition of adenylyl cyclase[21,22].

Serum Anticholinergic Activity and Anticholinergic Burden

M receptor antagonists have limited therapeutic use and are predominantly bladder antispasmodics used to treat urinary incontinence. Many other medications have anticholinergic properties despite the M receptor not being the intended receptor for effect[23,24]. Such agents tend to have a lower level of anticholinergic activity. However, when multiple drugs with low levels of anticholinergic activity are combined the cumulative anticholinergic activity and anticholinergic burden increases[23,25–27].

Anticholinergic activity is dependent upon many factors, including: the drug's binding to the M receptor, its absorption and distribution to tissues (including the brain), its concentration in circulation, intestinal and hepatic cytochrome P450 (CYP) metabolism and drug transport, the presence of any active metabolites that are produced, and the rate of elimination of the parent drug and active metabolites from the body. Since pharmacokinetics can be affected by sex, age or genetic polymorphisms (CYP enzymes) these all must be understood to quantify total anticholinergic activity and rationalize the use of anticholinergic medications in clinical practice.

Sex

Sex differences in pharmacokinetics have been explored with respect to some anticholinergic medications. Results of studies that examined sex differences in anticholinergic drug pharmacokinetics as their primary objective are listed in table 2.

Effect of sex on the absorption of anticholinergic medications

Some, but not all, studies show that gastric and colonic emptying is slowed in women, potentially increasing oral bioavailability of drugs[28–34]. When stratified by age, the rate of gastric emptying for postmenopausal women and men is similar[35] and significantly faster than premenopausal (younger) women[33]. Gastric pH is higher in females[36] which may increase absorption of basic medications such as tricyclic antidepressants, many of which are quite potently anticholinergic. This difference in gastric pH was quantified by Feldman and Barnett in 1991 as a mean pH of 2.79 for women and 2.16 for men, which was due to reduced acid secretion in women[37]. The greater stomach size in men allows for more fluid to be contained therein which can improve both the rate and extent of dissolution of introduced oral dosage forms for men in comparison to women. In contrast, intestinal pH has not been found to differ by sex[38,39]. CYP enzymes exist in intestinal enterocytes, where they contribute to the first pass metabolism of orally administered drugs. Intestinal CYP3A4 metabolism inconsistently exhibits sex differences. Early reports suggested that the CYP3A4 substrates verapamil and midazolam had increased bioavailability in women[40–42]. However, in 2005 a detailed analysis of duodenal punch biopsies from 48 men and 45 women found no clinically meaningful sex difference in intestinal CYP3A4 content[43]. Krecic-Shepard *et al.* observed that oral verapamil was cleared more quickly in men with no significant difference after IV administration, suggesting some differences in intestinal metabolism exist[44] which could affect those anticholinergic medications that are substrates of CYP3A4. In females, the CYP3A4 content in the intestine has been shown to decrease by approximately 20% after menopause[43] which may reduce CYP3A4 metabolism and affect the sex-difference in CYP3A4 pharmacokinetics in older women. This decrease in intestinal CYP3A4 in postmenopausal women has not been shown to be clinically meaningful to date. Similarly, male versus female differences in the drug efflux pump ABCB1 (p-glycoprotein) in the intestinal lumen have been hypothesized as a contributor to differences in drug absorption between sexes[43] but this too has not been demonstrated to be clinically meaningful in studies to date.

Effect of sex on the distribution of anticholinergic medications

In general, males are larger than females across the lifespan, with increased height, body mass index and waist circumference. Comparatively, women have increased adiposity[45]. This difference in body composition has failed to show much difference in actual drug distribution and any differences attributable to this can largely be explained by differences in total body mass[46]. Distribution of drugs to the brain is dependent upon the lipophilic nature of the blood-brain barrier which favours passage and accumulation of lipophilic drugs. At this time, no statistically significant difference has been found between similarly aged women and men with respect to albumin permeability of the blood brain barrier[47] which likely can be extrapolated to at least some medications. The brain is also protected by p-glycoprotein, which prevents drugs from accumulating in the brain by pumping them from brain capillary endothelial cells to the blood[48]. These mechanisms have not demonstrated any sex difference.

Effect of sex on the metabolism and transport of anticholinergic medications

Several studies support that hepatic CYP metabolism varies between men and women although the clinical significance is a challenge to understand fully. The most abundant hepatic CYP enzyme is CYP3A4 and it is involved in the metabolism of some anticholinergic medications. In humans CYP3A4 has a higher level of protein expression in the female liver[49]. Consistent with the expression data, CYP3A4 oxidation was reported to be more efficient in women[40,50] with a two-fold higher CYP3A4 hepatic content and 50% increase in the metabolizing capacity[51] but this finding has not been replicated in other scientific investigations[52,53]. An *in vitro* study from samples of 43 healthy livers in subjects between the ages of 27 and 83 showed a 24% increase in CYP3A4 activity identified by erythromycin N-demethylation in females[54]. Women have an increase in

CYP3A4 activity measured as a greater clearance of CYP3A4 substrates such as the weakly anticholinergic antihypertensive medication nifedipine[55] and the weakly anticholinergic sedative alprazolam[56]. On average, the weight-normalized clearance of alprazolam and nifedipine is mainly due to CYP3A4 and is 20% to 30% higher in young women than in young men. This difference applies to both parenteral and oral administration and is not explained away by p-glycoprotein activity[57]. For context, CYP3A4 activity has been studied in relation to metabolism of some non-anticholinergic agents, such as midazolam and clindamycin. Meta-analysis suggests that women exhibit a 16% higher weight-corrected oral clearance of midazolam ($p < 0.001$) and 20% higher systemic clearance ($p = 0.002$) than men. No significant difference in the area under the curve (AUC) after oral dosing of midazolam is found but after intravenous (IV) administration women showed lower AUC than men ($p = 0.02$). No sex-dependent differences were observed in midazolam bioavailability[58]. Clindamycin does not show any sex difference in its oral pharmacokinetics[59]. The study of midazolam and clindamycin confirm sex variability in CYP3A4 metabolism but fail to demonstrate any consistent sex-differences. In investigations of sex-differences in CYP2C19 activity, 4-hydroxymephenytoin and zonisamide failed to show any sex-differences[60,61]. A Spanish study found higher CYP2D6 activity in women[62].

Sex differences have been demonstrated in the glucuronidation of some medications (acetaminophen) but not others (zidovudine)[63–65], suggesting that sex differences in drug conjugation exist and are drug-dependent. To date, no anticholinergic agents have been explored with respect to glucuronidation. Clearance of some non-anticholinergic drugs by glucuronidation have been shown to be increased in men in comparison to women including oxazepam[50], temazepam[66] and acetaminophen[67]. With regard to catechol-O-methyltransferase activity, liver tissue from female subjects exhibited approximately 25% lower activity than samples from male subjects[68]. There is a two-fold greater expression of hepatic p-glycoprotein in men compared to women[69] with unclear clinical relevance.

Effect of sex on the renal elimination of anticholinergic medications

Kidney function is integral to drug elimination. Glomerular filtration is related to body mass. Males typically have a greater body weight than females[45], so generally glomerular filtration is greater in males than females. This likely explains the majority of sex-differences in renal drug clearance, though this has not been observed for all drugs. Sex has been found to be a significant factor in methotrexate clearance, with a 17% reduction in females after standardizing doses for body weight[70]. Some authors have reasoned that for narrow therapeutic index drugs the sex-related effect on kidney function may be clinically relevant[70,71]. Pharmacokinetic studies have confirmed sex-differences in renal clearance for many drugs including the weakly anticholinergic drug digoxin, which has slower clearance in females[72] and the moderately anticholinergic drug amantadine, which has been shown to have significantly higher renal clearance in men due to putative sex differences in renal tubule secretion by organic cation transporters[73].

Summary of Studies Showing Sex-Differences in Pharmacokinetics:

Quinidine

The most commonly reported anticholinergic medication with a focus on sex-related differences was quinidine, exploring drug induced QT interval prolongation (table 2)[74–76]. The findings of both Benton and Vicente[74,76] suggest that women clear quinidine at a faster rate than men. Unexpectedly, women have a more rapid onset of ECG changes in response to drug activity than men, which is not entirely explained by increased quinidine clearance. These studies demonstrate sex-differences in quinidine pharmacokinetics, however the mechanism of this difference is not clear[74–76]. It is possible hormonal influences or rapid distribution after IV infusion contribute to the faster onset of activity in women which normalizes over time to reach equilibrium between the sexes.

Summary of Studies Showing Sex-Differences in Pharmacokinetics: Psychoactive Medications

Many anticholinergic psychoactive medications have been investigated for sex-differences in absorption, distribution, metabolism and excretion. A study of cyclobenzaprine examined sex-differences using a series of open-label, three-period, randomized, crossover studies. The first study included 24 healthy young subjects

(mean age: 25.5 years), the second 18 healthy subjects (mean age: 28.7 years), and the third 12 older subjects (mean age: 71.3 years). The primary objective was to investigate the pharmacokinetics and bioavailability of cyclobenzaprine with attention to the effects of sex, age and hepatic insufficiency (table 2). There were small significant differences in the area under the curve (AUC) and C_{MAX} between sexes in the older group[77]. This is most likely due to accumulation of drug in the group of older females. A study of the benzodiazepine diazepam demonstrated a shorter $t_{1/2}$ and a greater plasma clearance in men in comparison to women (table 2)[78]. In a population of men and women receiving olanzapine for Alzheimer's Disease (AD) or schizophrenia, between one and six samples were analyzed from each individual to determine sex-differences in olanzapine clearance. Sex was found to be responsible for 12% of variability in olanzapine elimination. Men cleared olanzapine 38% faster than women[79]. A natural pharmacokinetic study of anticholinergic antidepressants in older adults looked for sex-differences in serum concentrations. The ratio of absolute serum concentration in comparison to the dose-adjusted serum concentration was 1.1-1.5-fold higher in women than in men for clomipramine and trimipramine. This was despite a dose reduction in females who received 10-30% lower dose but still achieved serum levels equivalent to male participants[80]. Findings of Mundo and Unterecker *et al.* suggest that clomipramine levels are not related to sex[81,82] but rather the metabolites of clomipramine accumulate contributing to the higher plasma levels seen in women. A second naturalistic study of antidepressants that examined 19,870 blood samples failed to show a difference for the tricyclic antidepressants clomipramine or fluvoxamine[83] which is in keeping with findings of Mundo and Unterecker[81,82]. However, in a study that examined dose regimens of fluvoxamine separately, a dose dependent sex difference in serum fluvoxamine concentration was observed. At a 100 mg daily oral dose, women achieved higher serum fluvoxamine concentrations than men, but with a 200 mg daily oral dose the serum concentrations were no longer statistically significantly different[84]. This may relate to a saturable metabolizing enzyme that is in a greater concentration or more active in men. Sex was correlated to paroxetine plasma concentrations in three studies that examined the effect of sex on paroxetine pharmacokinetics. In a study of 171 subjects aged [?]70 years, men had a higher paroxetine V_d (461$\pm$ 260 L) compared to women (346 $\pm$ 256 L)[85]. In a study of 1,677 older men and women the serum concentration of paroxetine was 32% higher in women (86 nmol/L versus 65 nmol/L, $p<0.001$)[83]. In a third study of 70 patients the plasma concentration of paroxetine was higher in women across age groups (28 versus 16 ng/mL; $p=0.001$)[86]. The mean AUC and C_{Max} for bupropion, a mildly anticholinergic antidepressant, were higher in women than men, however once these parameters were standardized for body weight the statistical significance was lost[87]. For bupropion, older women had a larger V_d and longer $t_{1/2}$ than young men. This does make it challenging to know how much of the effect was attributable to sex versus age[88]. Amitriptyline plasma levels were higher in women in a study of 110 inpatients receiving routine doses of amitriptyline[89], but no significant sex-difference in serum concentration of amitriptyline was noted in the study by Reis *et al.* [83]. Nortriptyline plasma levels were affected by sex with females experiencing higher plasma levels[90]. Desipramine was shown to have a longer elimination $t_{1/2}$ and a faster oral clearance in older men than in older women[91]. When examining risperidone plasma concentrations, the only parameter to exhibit a statistically significant difference between males and females was the plasma concentration/dose ratio. When weight was used to adjust the plasma concentration any difference was lost[92]. Many of these psychoactive medications are metabolized by CYP2D6 and a sex-related difference in CYP2D6 activity has not consistently been identified in the literature[93] which means there are likely other sex-dependent mechanisms contributing to these pharmacokinetics differences. In summary, while many sex-differences exist in the pharmacokinetics of psychoactive anticholinergic medications, the clinical relevance is unclear. The small increases in drug exposure that were identified (most often by women) may help explain the increased experience of adverse events by women[94,95].

Summary of Studies Showing Sex-Differences in Pharmacokinetics: Bladder Anticholinergics

Oxybutynin is the prototype bladder anticholinergic. Oxybutynin is metabolized by CYP3A4 to N-desmethyloxybutynin, which is considered to cause many of the adverse events related to oxybutynin treatment. Increased CYP3A4 activity and slowed renal elimination in women may increase exposure to the metabolite and increase the likelihood of adverse drug effects. However, an older study of oxybutynin pharmacokinetics failed to show sex differences in the pharmacokinetics of oxybutynin or its metabolite[96].

Two randomized double-blind placebo-controlled trials assessed the effects of age, sex and race on the pharmacokinetics, pharmacodynamics and safety profiles of fesoterodine in 32 healthy males aged 18-45 years and 16 young men, 16 older men and 16 older women (table 2). Total plasma clearance of fesoterodine was highest in young men and lowest in older women but there were no apparent sex differences in C_{Max}, $AUC_{0\text{-}[?]}$, or t_{max}. Interestingly five hours after the dose was given, older women experienced a one gram decrease in salivary volume whereas older men did not, which provided some evidence that women are more likely to experience adverse effects (e.g. dry mouth) from anticholinergic medication use[97]. Similarly, in a study of 337 individuals darifenacin clearance was about 30% lower in females[98]. No sex differences in pharmacokinetics have been identified for solifenacin[99] or tolterodine[97]. Trospium demonstrates an unexplained prolonged $t_{1/2}$ in women compared to men[100]. This demonstrates the complex influence of sex on pharmacokinetics of bladder anticholinergics which are frequently used by older adults.

Summary of Studies Showing Sex-Differences in Pharmacokinetics: Antihistamines

A single-centre, single dose, open-label, reference replicate, bioavailability study in 12 healthy males and 12 healthy females aged 18-45 years with a body mass index between 19-30 kg/m^2 was completed to determine the effect of sex on the pharmacokinetics of doxylamine–pyridoxine 10 mg–10 mg delayed-release tablets. Females had significantly larger $AUC_{0\text{-}t}$ and a higher C_{Max}, for doxylamine compared to males[101].

Summary of Studies Showing Sex-Differences in Pharmacokinetics: Scopolamine

An open label crossover study of 7 men and 7 women of mean age 23 years and in good health was completed to identify any sex differences in pharmacokinetics in the metabolism of 0.5 mg scopolamine when given IV or orally with or without grapefruit juice. The C_{Max} was significantly higher in males than females (6.61 ng/mL versus 3.93 ng/mL) after IV infusion with all other parameters being similar[102]. No sex differences were found in urinary elimination of scopolamine for any of the three different routes of administration.

Age

Effect of age on the absorption of anticholinergic medications

Changes experienced by the aging body are another potential contributor to changes in drug pharmacokinetics. Studies with a primary objective of identifying age-related differences in drug pharmacokinetics are listed in table 3. Gastric and colonic transit is significantly faster in postmenopausal women in comparison to premenopausal women[33] which alters absorption. In a study of 16 healthy adults average age 81 years and 16 healthy adults average age 24 years, advanced age did not influence gastric emptying or small intestinal transit but that older individuals had a slower colonic transit[29].

Effect of age on the metabolism and transport of anticholinergic medications

In humans, it is well established that total hepatic CYP enzyme levels decline from about age 40 onwards. This has been quantified as about a 3.5% decline in CYP enzyme content for each decade of life potentially influencing the elimination of anticholinergic drugs undergoing metabolism by the CYP enzyme system, resulting in greater exposure to the pharmacologic properties of these agents[52,103]. An older study investigating the metabolic ability of CYP 450 enzymes in aging revealed that CYP3A4 was reduced in older adults. The microsomal content of CYP3A4 was found to decrease by approximately 8% per decade of life[52]. This trial failed to show a difference in CYP1A2, or CYP2C based on aging. An in vitro study of healthy human liver samples obtained during surgical procedures from 43 subjects between the ages of 27 and 83 showed no variation in CYP3A4 activity in relation to age. In this study, CYP3A4 activity was quantified by measuring erythromycin N-demethylation. While erythromycin N-demethylation has been shown to decline with age, the results of this study suggests that the age-related decline in enzyme activity is not due to declining CYP3A4 activity. Rather, other patient factors such as renal blood flow, renal filtration or body composition are likely contributing[54]. In females intestinal CYP3A4 content has been shown to decrease by approximately 20% after menopause[43] which may reduce intestinal CYP3A4 metabolism and

contribute to an age-dependent difference in CYP3A4 metabolism. Possibly due to a lack of studies, this decrease in intestinal CYP3A4 in postmenopausal women has not been shown to be clinically meaningful to date. Decreases in the clearance of CYP3A4 substrate drugs suggest that older people may experience increased adverse effects due to reduction in clearance of drugs that rely on CYP3A4 for metabolism prior to elimination[104].

Drug Conjugation has been shown in several studies as remaining fairly constant with respect to age[105]. Undeniably, numerous factors such as genetics, medication use and frailty[106,107] can influence glucuronidation and sulfonation but in younger and older healthy people glucuronidation and sulfonation are not statistically significantly different. In aging rat models, liver sinusoidal endothelial cells undergo pseudocapillarization[108,109] a process characterized by loss of sinusoidal fenestrations, thickening of the endothelium, perisinusoidal collagen deposition and basal lamina formation[110]. This process suggests that drug passages through the liver are reduced in size which in theory could prevent large molecules, in particular protein therapeutics and extensively protein bound drugs, from travelling through the liver and being cleared; this has been shown for liposomal doxorubicin in aged*vs.* young rats[111]. The relevance of these changes to anticholinergic drug pharmacokinetics remains to be determined

Effect of age on the renal elimination of anticholinergic medications

Renal elimination declines with age by all renal routes (glomerular filtration, tubular secretion, and passive reabsorption)[112,113]. Any anticholinergic agent that is renally eliminated or has a renally eliminated active metabolite is likely to accumulate in older adults in comparison to younger adults.

Effect of age on Blood-brain barrier function

In men the V_d of (R)-[11C] verapamil, a known p-glycoprotein substrate, increased with age in several cortical brain regions, strongly suggesting a progressive decrease in blood brain barrier p-glycoprotein function with age[114]. This could affect drug introduction to the brain which may affect efficacy or toxicity depending upon the agent used.

Summary of Studies Showing Age-Differences in Pharmacokinetics: Psychoactive Medications

Risperidone is a commonly used antipsychotic agent with anticholinergic properties. Both risperidone and its 9-hydroxyrisperidone metabolite are active. In a study of 129 adults on risperidone maintenance therapy grouped by age (<45, 45-60 and >60 years), the risperidone maintenance dose was lowest in the oldest age group, but the unadjusted plasma risperidone concentrations did not differ significantly across age groups. However, when adjusted for subject body weight or maintenance dose the plasma risperidone concentration was significantly higher in the older group. The concentration of active drug was comprised of both the 9-hydroxyrisperidone metabolite and risperidone parent drug, with the difference driven by the 9-hydroxyrisperidone concentration[115]. This supports the use of the lowest dose possible of risperidone in older adults and provides support for a "start low and go slow" approach to antipsychotic dosing in geriatric populations. In comparison, the clearance of the sedative diazepam was not found to be affected by age in a study of young (21-30 years) males and females in comparison to older males and females (70-88 years)[78]. A naturalized study of multiple anticholinergic antidepressants showed an increase in the absolute serum concentrations to dose adjusted serum concentrations for fluvoxamine (2-fold), amitriptyline and clomipramine (1.5-fold) in the oldest age group more than 65 years of age in comparison to controls less than 40 years. No significant age difference was observed for the dose adjusted fluoxetine and trimipramine serum concentrations. For fluoxetine and trimipramine users, older adults were using 10-30% lower total daily doses. The concentration to dose ratio of nortriptyline was two-fold higher in adults over 65 in comparison to the controls less than 40 years old [80]; clearance was correlated with age with faster clearance at younger ages. No significant difference was found between patients younger or older than 60 years in the mean dose-corrected serum concentration of clomipramine and N-clomipramine, which contradicts the findings of Waade[82]. However amitriptyline plasma levels were higher in older adults than younger subjects[89] which was consistent with findings of Waade*et al.* and Dawling *et al* . who showed that both amitriptyline and nortriptyline levels were higher in older adults with older women experiencing a more exaggerated effect

than their male comparators[116]. At daily oral doses of 100 mg or 200 mg, fluvoxamine serum concentration did not correlate with age[84]. There was a trend to higher serum concentrations in older female patients with the lower dosage, but this diminished when the dosage was doubled and suggests there is an interaction between age and sex on fluvoxamine pharmacokinetics. Older subjects taking oral paroxetine had higher plasma concentrations than younger subjects[117]. In a study that examined bupropion kinetics in older adults with depression (mean age 71.5 years) clearance was 80% of that seen in younger adults[88]and $t_{1/2}$ was 34 hours in comparison to most sources which report 11-14 hours[88,118]. Among females, there was no significant difference between young and older groups in any of the pharmacokinetic variables for triazolam. Among males, the $t_{1/2}$ of triazolam increased. Furthermore, when age was evaluated as a continuous variable, AUC increased significantly with age ($p = 0.02$) and clearance decreased with age ($p = 0.02$). Further examination of cyclobenzaprine pharmacokinetics showed increased $t_{1/2}$ in older *vs.* younger adults[77].

Summary of Studies Showing Age-Differences in Pharmacokinetics: Bladder Anticholinergics

The potently anticholinergic drug oxybutynin follows the trend of increasing peak plasma levels and bioavailability with increasing age and frailty[119]. This effect is so significant that study authors suggested halving the dose of oxybutynin so older adults to achieve the same plasma levels as younger adults. AUC and C_{Max} are increased 20% and 16% respectively when an older population is given the same dose of oxybutynin as a younger population. Moreover, solifenacin, a newer bladder anticholinergic, has a longer $t_{1/2}$ due to slower elimination and longer time to reach C_{Max} in older adults. This can be explained by the slowed absorption of solifenacin in older adults which increases their exposure to solifenacin by about 1.2-fold[99]. In a study of 16 young men, 16 older men and 16 older women, receiving either 8 mg of fesoterodine extended release or matching placebo, the renal clearance of fesoterodine was 28% lower in older men and women than younger men[97] (table 3). This increased exposure to fesoterodine in older adults may predict increased exposure of tolterodine in older adults as well, as fesoterodine and tolterodine are related compounds, with both being metabolized to the same active ingredient.

Summary of Studies Showing Age-Differences in Pharmacokinetics: Scopolamine

Healthy adult subjects were given scopolamine hydrobromide 0.5 mg IV if they were under 65 years of age and 0.3 mg if older than 65 years. These subjects then received a battery of tests of cognitive function in addition to measurement of pharmacokinetic variables. Older age was associated with slowed clearance and increased exposure to scopolamine. Age-related increases in scopolamine exposure was likely the greatest contributor to the increased sensitivity to cognitive adverse effects in older adults. The study authors hypothesized that age-related changes in CYP3A4 activity or content may have been responsible for the increased scopolamine exposure in older adults[120].

Genetics

In addition to age and sex, it is important that we understand how genetic variation in CYP activity can influence clinical effect or toxicity as drugs that are substrates for these enzymes are frequently used by older adults[15].

CYP2D6

Genetic variation in the *CYP2D6* gene is well characterized and gives rise to at least 120 *CYP2D6* variants (alleles) that have altered levels of CYP2D6 enzyme activity. These alleles result from point mutations, deletions or additions, gene rearrangements and deletion or duplication/multiplication of the entire gene and have different distribution among various ethnic groups. Phenotypically, individuals with two normal CYP2D6 alleles are extensive metabolizers (EMs), those with one normal and one poor metabolism allele are intermediate metabolizers (IMs) and those with 2 reduced metabolism alleles are poor metabolizers (PMs). For CYP2D6, there is a fourth phenotype, the ultra-rapid metabolizers (UMs) who have at least one CYP2D6 gene duplication. Of interest, PM variants are common in East Asian populations and exist

across the world. Understanding the effect of these CYP2D6 variants on pharmacokinetics is important for predicting drug effect and adverse effect.

The effect of CYP2D6 phenotype on anticholinergic medication exposure has been investigated in older adults. CYP2D6 phenotypes have been well characterized with respect to codeine pharmacokinetics and pharmacodynamics. Limited activation and effect of codeine occurs in CYP2D6 PMs and increased metabolism and toxicity has been reported in UMs[121]. Nortriptyline plasma levels were mostly correlated to CYP2D6 genotype and sex[90]. In nursing home patients exposed to anticholinergic drugs the highest serum anticholinergic activity was found in groups of CYP2D6 PMs[122]. Analysis of risperidone metabolism in 70 healthy volunteers (of whom 82.9% were either IM or EM) revealed that polymorphisms of the CYP2D6 enzyme were much more responsible than sex for variation in risperidone metabolism. CYP2D6 phenotype explained 52% of interindividual variability in risperidone pharmacokinetics. The AUC of the active moiety was found to be 28% higher in CYP2D6 PM compared with IM, EM and UM. No other genetic markers were found to significantly affect risperidone concentrations[123]. This genetic variation in the metabolism of risperidone is of such magnitude that it could alter results when conducting bioequivalence studies[124]. Differences in dose responses should be considered as clinically relevant for any person initiated on risperidone, further supporting using the lowest possible doses at all times.

The bladder anticholinergic tolterodine is metabolized to a similarly active 5-hydroxymethyl tolterodine (5-HMT) by CYP2D6. The bioavailability of tolterodine is strictly related to the genetic polymorphism of CYP2D6 and it ranges from 10% to 74%[125]. Byeon *et al.* investigated the relationship between CYP2D6 phenotypes and tolterodine pharmacokinetics in 46 Korean subjects. The single dose and multiple dose C_{Max} and $AUC_{0\text{-}24}$ of tolterodine was significantly higher in the PM groups than in the EMs. The ratio of clearance to bioavailability of tolterodine in the EMs was 5 to 18-fold higher than PM (variant dependent) in multiple dosing studies[126]. A Swedish study also found a difference in the absorption $t_{1/2}$ of tolterodine between EM (0.41 h) and PM (0.53 h) and EM were found to have a slight increase in heart rate at steady state in comparison to baseline which was thought to be related to drug exposure[127]. Interest in understanding drug induced QT interval prolongation led to study of the effect of CYP2D6 polymorphism on ECG changes in the use of tolterodine and its active metabolite 5-HMT. In CYP2D6 PM the systemic exposure to tolterodine is higher than EM ($t_{1/2}$ of tolterodine IR 10 h in PM versus 2-3 h in EM) which may contribute to differences in ECG changes[127]. However, the total concentration of active moieties (tolterodine plus 5-HMT) was similar for PM and EM which makes dose adjustment unhelpful for equalizing drug exposure. Interestingly, 5-HMT and tolterodine may contribute differently to QT interval prolongation risk and so this was studied as well. QT interval prolongation in CYP2D6 PM was only slightly greater for PM likely due to differences in protein binding between the two active components[128]. As a further illustration of the impact of CYP2D6 genetic variation on anticholinergic pharmacokinetics, 4 mg daily dosing of fesoterodine produced a C_{Max} of 3.45 ng/mL in CYP2D6 PM versus 1.89 ng/mL in CYP2D6 EM. A similar proportional result was also observed for 8 mg daily dosing of fesoterodine in PM (C_{Max} of 6.40 ng/mL) versus EM (C_{Max} 3.98 ng/mL). Fesoterodine equally follows CYP2D6 and CYP3A4 metabolism which should make it less susceptible to CYP2D6 reduced metabolism but this has not been clearly demonstrated[129]. The oral antimuscarinic agent darifenacin is metabolized by CYP3A4 and CYP2D6 with the main metabolite being inactive[130]. The oral bioavailability of darifenacin is significantly altered by the CYP2D6 genotype in a dose-dependent fashion. In EM the bioavailability of 7.5, 15 and 30 mg CR oral doses of darifenacin are 15%, 19% and 25%, respectively. In IM and PM this bioavailability becomes 40 to 90% higher. There is less impact of the CYP2D6 variants on the systemic elimination of darifenacin. In UM the $t_{1/2}$ of darifenacin is 3.12 h, while in PM it is 3.83 h[98].

All told, CYP2D6 is an important contributor to variation in pharmacokinetics of medications it metabolizes. In a study of patients with schizophrenia, Jurgens et al. reported that PM and UM did receive higher doses of medication, including CYP2D6 dependent antipsychotics, than EM and IM. UM would likely need higher doses to compensate for their increased metabolism, so it is reassuring to see this in practice. However higher doses being used by PM may reflect adverse drug events being misinterpreted as psychotic symptoms leading to inappropriate and potentially harmful dose increases [131].

CYP2C19 and CYP3A4

Genetic polymorphisms in the CYP2C19 gene also result in PM, IM and EM phenotypes. To date no studies have demonstrated a role of CYP2C19 genetic variation in anticholinergic medication pharmacokinetics. Previous research has failed to identify individuals with no CYP3A4 activity. Due to the lack of genetic PM of CYP3A4, other factors such as exposure to drug inducers and inhibitors, liver function, blood flow, and possibly age and sex are the biggest considerations for variation in CYP3A4 activity[49,54].

Conclusions

Anticholinergic medications pose health risks to older adults. We know that adverse drug reactions due to anticholinergic medications are most commonly proportional to plasma drug concentrations or serum anticholinergic activity[12,13,132] which makes sex, age, and genetic effects on drug disposition relevant for clinical decision making. Investigating the role of sex, age and CYP polymorphism on anticholinergic medications confirms that women often experience increased drug exposure[32,101] which likely contributes to their experience of more adverse drug reactions than men[74,76,94,95,97] and increasing age can also increase drug exposure. There may be a role for differential dosing of some drugs based on age and sex. Clinical testing of CYP2D6 polymorphisms and adoption of peer-reviewed published clinical practice guidelines for prescribing based on genotype where strong evidence exists may also help reduce the burden of adverse drug responses in older people.

The take home message is that the greatest increase in drug exposure is likely experienced by older women. Clinical practice demonstrates that even a small decrease in dose modestly decreases adverse drug reactions with negligible effect on efficacy which should encourage clinicians to minimize anticholinergic drug doses, if anticholinergic medications must be used at all. While the tenants of Geriatric medicine have been relatively effective in communicating the importance of lower doses in older adults, the importance of sex in dosing has been poorly translated into clinical practice. Monographs frequently provide advice for dosing in the oldest users but rarely offer advice for dosing in women. With increased risk of hospitalization, cognitive impairment and mortality as risks from anticholinergic drug use, improved understanding of sex, age and genomic testing of CYP isozymes may be indicated to reduce serious anticholinergic adverse events. Rigorous pharmacokinetic analysis is a much needed and important next step to allow us to understand how dosing recommendations can be modified to most safely and effectively treat older men and women. Studies that have been done in the past often examined age sex or CYP polymorphisms alone and future work needs to account for all of these factors so that we may better approach personalized medicine for optimal outcomes.

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Table 1: Studies that have investigated the risk of serious adverse drug events and mortality related to the use of anticholinergic drugs in older adults

Study Authors/Design	Study Population	Study Objective	Drug Measure	Adverse Event

Study Authors/Design	Study Population	Study Objective	Drug Measure	Adverse Event
Myint et al[10] Cohort Study	25,639 men and women 40-79 years old from general practice registers in the EPIC-Norfolk cohort, UK followed for >10 years	To examine the relationships between total anticholinergic burden, all-cause mortality, and cardiovascular disease	Total Anticholinergic Burden Score using an Anticholinergic Burden Scale	Higher rates of all-cause mortality and cardiovascular disease in group with higher anticholinergic burden score
Hilmer et al[6] Cohort Study	3,075 community-dwelling Medicare recipients aged 70-79 years, recruited from April 1997 to June 1998 in eastern USA	To determine if total drug burden exposure over 5 years is associated with reduced functional capacity at year 6	Drug Burden Index (DBI) (calculated based on anticholinergic agents and sedative use)	Higher DBI at years 1, 3 and 5 was consistently associated with poorer function at year 6; with a reduction in gait speed and grip strength
Chew et al[132] Cohort Study	35 inpatients in a geriatric inpatient ward between February 2000 and April 2002 with behavioural and psychological symptoms of dementia (BPSD)	To examine association between serum anticholinergic activity (SAA) and cognitive performance in patients with moderate-to-severe dementia	SAA as measured by a radioreceptor competitive binding assay	Moderate negative correlation between SAA and Mini-Mental State Examination (MMSE) score
Golinger et al[12] Cohort Study	25 patients in the surgical intensive care unit over a 3-month period	To determine presence of delirium and to estimate risk of delirium using SAA	Assay for SAA was performed on each sample by a radioreceptor method	The mean SAA was significantly higher for the delirious (4.67±3.3 ng/mL), versus non-delirious (0.81±1.0 ng/mL) patients

Study Authors/Design	Study Population	Study Objective	Drug Measure	Adverse Event/Outcome

Study Authors/Design	Study Population	Study Objective	Drug Measure	Adverse Event
Mulsant et al[13] Cohort Study	201 randomly selected from a cohort of English-speaking adults [?]65 years who had serum samples collected between March 1995 and September 1997	To examine the relationship between SAA and cognitive performance in a cohort of community dwelling individuals	SAA as measured by a radioreceptor method	SAA was strongly associated with cognitive impairment and those participants with SAA higher than the 90th percentile were 13 times more likely to have an MMSE score below the 10th percentile than participants with an undetectable SAA
Han et al[5] Cohort Study	Inpatients [?]65 years of age with delirium, admitted to medical or geriatric services	To evaluate the association between use of anticholinergic medications and severity of delirium	Anticholinergic medication use calculated by: Summer's Drug Risk Number Clinician-rated anticholinergic score Number of anticholinergic medications Number of non-anticholinergic medications Total number of medications	Increase in delirium severity was significantly associated with the clinician-rated anticholinergic score and the number of anticholinergic medications for both those with and without baseline dementia

Study Authors/Design	Study Population	Study Objective	Drug Measure	Adverse Event/Outcome
Haab et al[133] Noncomparative, open-label study	719 adults (85.1% women), mean age 57.3 years, with 29.9% aged [?]65 years who had completed a feeder darifenacin study	The primary objective was to assess the long-term safety, tolerability and efficacy of darifenacin in patients with overactive bladder	Darifenacin controlled release 7.5 or 15 mg orally once daily	The most commonly reported adverse events were dry mouth and constipation, for which the all-causality incidence was 23.3% and 20.9%, respectively with 0.4% of patients reporting hypertonia, somnolence and paresthesia

Study Authors/Design	Study Population	Study Objective	Drug Measure	Adverse Event
Ancelin et al[3] Cohort Study	372 elderly participants without dementia aged >60 years randomly selected from 63 general practitioners in the Montpellier region of southern France	To assess the potential of anticholinergic drugs as a cause of non-degenerative mild cognitive impairment in elderly people	From an extensive literature review a table was created associating known anticholinergic drugs with their SAA and participant's records were examined to classify the anticholinergic burden from 0 to 3	Anticholinergic drug users had poorer simple reaction time, attention, immediate and delayed visuospatial memory, narrative recall, verbal fluency, object naming, and visuospatial construction and anticholinergic drug use was a significant predictor of mild cognitive impairment (OR 5.12; 95% CI[1.94 to 13.51])
Study Authors/Design	**Study Population**	**Study Objective**	**Drug Measure**	**Adverse Event/Outcome**
Lechevallier-Michel et al[9] Cross-sectional Study	3,777 subjects among French elderly [?]65 years, living in the community in two administrative areas in south western France	The aim of this study was to assess the association between the use of drugs with anticholinergic properties and cognitive performance among community-dwelling elderly subjects	Anticholinergic drugs from seven therapeutic classes were examined: antihistamines, gastrointestinal and urinary antispasmodics, antiemetics, bronchodilators, antiparkinsonian drugs, antidepressants and antipsychotics	Current use of anticholinergic drugs was significantly associated with low cognitive performance on cognitive tests (MMSE, BVRT, IST) among community-dwelling elderly people
Geller et al[134] Cohort Study	50 cognitively intact women aged [?]55 years seeking treatment for overactive bladder	To investigate the effect of trospium chloride extended release, on cognitive function in postmenopausal women in a clinic setting	Hopkins Verbal Learning Test (HVLT-R) assessed at day 1, at week 1, 4 and 12 of treatment with trospium chloride	At week 1 there was a decline in the HVLT-R learning subscale (p=0.029), at week 4 the HVLT-R Total Recall subscale score was improved over baseline (p=0.02)

Study Authors/Design	**Study Population**	**Study Objective**	**Drug Measure**	**Adverse Event**
Hill et al[135] Noncomparative, open-label study	716 patients aged [?]65 years with overactive bladder who had first completed 12 weeks of a feeder study	To determine the long-term safety, tolerability and efficacy of darifenacin in patients [?]65 years of age	darifenacin 7.5 mg once daily for 2 weeks then a dose increase to 15 mg once daily with monitoring of safety, tolerability and efficacy	Dry mouth and constipation led to discontinuation in 2.3 and 4.2% of participants respectively, cardiovascular and peripheral/CNS adverse events were infrequently reported; 1.4% and 3.3% respectively
Study Authors/Design	**Study Population**	**Study Objective**	**Drug Measure**	**Adverse Event/Outcome**
Armstrong et al[136] Pooled data from 2 multicenter, randomized, double-blind, parallel group trials	1,168 patients [?]18 years of age with a diagnosis of overactive bladder, as defined by urge urinary incontinence, urgency and frequency	To describe the safety and tolerability of extended-release oxybutynin at 10 mg once daily and to compare the safety profile with that of tolterodine 4 mg once daily	Extended-release oxybutynin 10 mg once daily, immediate-release tolterodine 2 mg twice daily and extended-release tolterodine 4 mg once daily were compared over 12 weeks	Approximately 10% of participants had one or more adverse events associated with the nervous system, with no clinically relevant differences across the three treatment groups (extended-release oxybutynin, 10.2%; extended-release tolterodine, 8.3%; and immediate release tolterodine, 10.9%)
Koyama et al[8] Cohort Study	4,606 women [?]65 years of age recruited in Minneapolis, Minnesota; Portland, Oregon; Baltimore, Maryland; or Monongahela Valley, Pennsylvania between 1986 and 1988	To determine whether anticholinergic load is associated with a higher risk of functional impairment and low cognitive performance	Anticholinergic load measured using the total score on the Anticholinergic Cognitive Burden scale (ACB)	A one-unit increase in ACB score was significantly associated with one or more new Instrumental Activities of Daily Living (IADL) impairments (OR 1.11; 95% CI [1.04 to 1.19]) and with worse cognitive performance
Study Authors/Design	**Study Population**	**Study Objective**	**Drug Measure**	**Adverse Event/Outcome**

Study Authors/Design	**Study Population**	**Study Objective**	**Drug Measure**	**Adverse Event**
Fox et al[4] Longitudinal Study	13,004 participants representative of the population aged [?]65 living at home or in institutions in England and Wales	To identify if the use of medications with possible and definite anticholinergic activity increases the risk of cognitive impairment and death in older people	Each participant's anticholinergic burden was calculated using the ACB	A dose-response relationship was observed between increased total ACB score and MMSE decline, with a score of 4 or more on the ACB associated with a 0.34 (95% CI [0.01 to 0.67]) lower MMSE score than those not taking anticholinergics and for each 1 point increase in ACB, the odds of dying increased by 26% (OR 1.26; 95% CI [1.20 to 1.32])
Kalisch Ellett et al[7] Cohort Study	36,015 subjects from the Australian veteran community, which includes veterans and war widows and widowers with median age 80	To examine the effect of use of anticholinergic medications on the risk of hospitalization for confusion, dementia, or delirium	The estimated daily number of anticholinergic medications were expressed as no medication or one, two, three or more anticholinergic medications	The risk of hospitalization was greater when using two (RR 2.58; 95% CI [1.91 to 3.48]), or three or more anticholinergic medications (RR 3.87; 95% CI [1.83 to 8.21]) than when participants were not exposed to anticholinergic medications

Table 2: Details of study population, study objectives, methodology and results of trials identified to have a primary objective of exploring sex-differences in pharmacokinetic parameters for anticholinergic medications

Study Author & Design	Study Population	Study Objective	Methodology	Results
Vicente et al[76] Randomized single blind controlled trial	24 healthy non-smoking volunteers (12 women and 12 men), 18-35 years old	To determine if quinidine induced prolongation of the time from the peak to the end of the T-wave is greater in women than men	Subjects received either 4 mg/kg of quinidine IV or a matching placebo solution over 20 min with 28 blood samples and simultaneous ECGs collected after drug/placebo infusion for each subject at predetermined time points over the following 12 h	- Quinidine causes QTc prolongation and T-wave morphology changes in both women and men - Quinidine-induced maximum QTc (541 ± 40 versus 510 ± 38 ms; p = 0.07) or maximum T_{peak}-T_{end} (216 ±60 versus 222 ± 37 ms; p = 0.76) was similar for men and women - There was a trend toward a lower maximum serum quinidine concentration in women compared to men (2.9 ± 0.7 versus 3.7 ± 1.2 μg/mL; p = 0.07) - The slope describing serum quinidine concentration versus QTc prolongation was greater in women than in men (38 ± 10 ms/μg/mL vs. 28 ± 9 ms/μg/mL; p = 0.02) - Differences between women and men occurred primarily in the first 20 min after quinidine infusion, when serum quinidine concentrations were higher in men than women
Study Author & Design	**Study Population**	**Study Objective**	**Methodology**	**Results**

Study Author & Design	Study Population	Study Objective	Methodology	Results
Benton et al[74] Randomized single-blinded controlled trial	24 healthy non-smoking volunteers (12 women and 12 men), 18-35 years old	To determine if women have larger increases in QT interval than men at equivalent serum concentrations of quinidine after intravenous administration	Subjects received either 4 mg/kg of quinidine IV or a matching placebo solution over 20 min. 28 blood samples and simultaneous ECGs were collected after drug/placebo infusion for each subject at predetermined time points over the following 48 h	- There was a trend to greater weight-adjusted clearance of quinidine in women than in men (5.2 ± 1.1 versus 4.3 ± 1.6 mL/min/kg) - There was also a trend to a higher maximal plasma concentration of quinidine in men than in women (3.67 ± 0.13 versus 2.78 ± 0.87 μg/mL; p = 0.07) - There were no sex-related differences in the ratio of the $AUC_{[?]}$ of 3-hydroxyquinidine to the $AUC_{[?]}$ of quinidine - The estimated volume of distribution (V_d) at steady state was not different between the men and women - There was no difference in the free fraction of quinidine in serum between men and women - The free fraction of 3-hydroxyquinidine was slightly higher in women than in men (0.53 ± 0.05 μg/mL versus 0.47 ± 0.05 μg/mL; p < .01)
Study Author & Design	**Study Population**	**Study Objective**	**Methodology**	**Results**

Study Author & Design	Study Population	Study Objective	Methodology	Results
Winchell et al[77] A series of open-label, three-period, randomized, crossover studies	1. 24 healthy young subjects (mean age: 25.5 years; range: 19-39 years; 16 males and 8 females 2. 18 healthy subjects (mean age: 28.7 years; range: 22-40 years; 8 males, 10 females) 3. 12 elderly subjects (mean age: 71.3 years; range: 65-79 years; 6 males, 6 females	To investigate the pharmacokinetics and bioavailability of cyclobenzaprine, including the effects of sex and age	1. Bioavailability: Subjects received 5 mg orally or 1.25 mg IV cyclobenzaprine 2. Pharmacokinetics: Subjects received a single oral dose of 2.5, 5, or 10 mg cyclobenzaprine on Day 1 then every 8 h from Days 8 through 14 with final dose on Day 15 3. Pharmacokinetics in aging: Subjects received 5 mg cyclobenzaprine orally three times daily for 7 days and a final dose on Day 8	1. Plasma concentrations increased initially, peaking at 4 h post dose, and then declined slowly - Mean plasma clearance was 689 ± 216 mL/min - - Mean oral bioavailability 5 mg tablet formulations were 0.55 (90% CI[0.51, 0.60]) 2. There were no statistically significant differences between males and females for any of the pharmacokinetic parameters - $AUC_{(0\text{-}8\ h)}$ and C_{Max} after the last dose were marginally significantly different between sexes 3. The population-by-sex effect was marginally significant for $AUC_{(0\text{-}8\ h)}$ ($p = 0.056$) but not for C_{Max}
Study Author & Design	**Study Population**	**Study Objective**	**Methodology**	**Results**

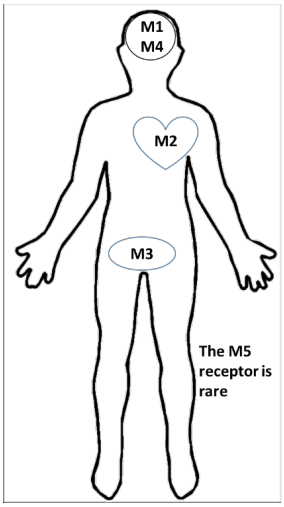
Study Author & Design	Study Population	Study Objective	Methodology	Results
El-Eraky et al[75] Open trial	48 healthy volunteers (27 men, 21 women) aged 18-64 years	To determine why women are more susceptible to QT interval prolongation and torsade de pointes after administration of drugs that delay cardiac repolarization	All subjects took quinidine sulphate capsules 3 mg/kg orally then ECGs and blood samples for quinidine concentrations were taken over 24 h following drug administration	- There were no significant differences in quinidine concentrations between men and women or in any of the pharmacokinetic variables measured - The QT_a, and QT_c intervals were larger in females than in males - Quinidine did not affect QRS duration in women but reduced QRS duration in men
Koren et al[101] Single-centre, single dose open-label, reference replicate bioavailability study	12 healthy males and 12 healthy females, 18-45 years with a body mass index between 19-30 kg/m^2	To determine the effect of sex on the pharmacokinetics of doxylamine–pyridoxine 10 mg–10 mg delayed-release tablets	Participants were given doxylamine–pyridoxine 20 mg–20 mg delayed-release tablets with 240 mL water on an empty stomach with blood sampling starting 1 h pre-dose with samples analyzed using high performance liquid chromatography-tandem mass spectrometry	- Females had significantly larger $AUC_{0\text{-}t}$ for doxylamine compared to males - A higher C_{Max} for doxylamine was observed in females compared to males
Study Author & Design	**Study Population**	**Study Objective**	**Methodology**	**Results**

Study Author & Design	**Study Population**	**Study Objective**	**Methodology**	**Results**
Malhotra et al[97] Two randomized double-blind placebo-controlled trials	1. 32 healthy males aged 18-45 years 2. 16 young men, 16 older men and 16 older women	To examine the effect of age, sex and race on the pharmacokinetics, pharmaco-dynamics and safety profiles of fesoterodine	Subjects received either 8 mg of fesoterodine extended release or placebo with blood samples drawn over 36 h after drug administration and saliva samples on cotton wool collected over 24 h after drug administration	- No apparent differences in C_{Max}, $AUC_{0\text{-}[?]}$, t_{max}, or mean residual time between males and females - Total plasma clearance was highest in young men and lowest in older women - Elderly women experienced a 1 g decrease in salivary volume and elderly men did not 5 h after dose - Elderly men experienced the greatest residual urinary volume increase 8 h after dose
Ebert et al[102] Open label crossover study	7 men and 7 women of mean age 23 years and in good health	To identify any pharmacokinetic differences between male and female volunteers in the metabolism of scopolamine when given with grapefruit juice	Each subject received at random scopolamine 0.5 mg IV, scopolamine 0.5 mg orally, or scopolamine 0.5 mg orally mixed with 150 mL fresh grapefruit juice and blood sampling occurred over the 24 h following drug administration	- C_{Max} was significantly higher in males than females (6.61 ng/mL versus 3.93 ng/mL) after IV infusion - All other parameters were similar
Study Author & Design	**Study Population**	**Study Objective**	**Methodology**	**Results**

Study Author & Design	**Study Population**	**Study Objective**	**Methodology**	**Results**
Macleod et al[78] Open label study	4 men and 5 women aged 21-30 years, and 5 older men and 5 older women aged 70-88 years	To identify age and gender differences in diazepam pharmacokinetics	10 mL blood samples were taken over 1 week after receiving 0.125 mg/kg diazepam IV over 10 minutes	- There was a significant difference in plasma clearance between men and women (male: 33.2 mL/min and women: 18.1 mL/min) - The half-life in men (32 h) was significantly shorter than in women (46.2 h) - V_d was not significantly different between sexes
Bigos et al[79] Naturalized prospective study	332 men and 191 women who were using olanzapine for AD or schizophrenia	To evaluate population pharmacokinetics of olanzapine and factors that contribute to variability in exposure including sex, race and smoking status	Plasma levels of olanzapine were determined and then used to calculate non-linear mixed effects modelling for pharmacokinetic analysis	- Men cleared olanzapine 38% faster than women ($p < 0.0001$, unpaired t test)
Hartter et al[84] Prospective study	15 male and female participants with major depression	To assess sex differences in fluvoxamine serum concentration at two different fixed dosing regimens (50 twice daily and 100 mg twice daily)	Drug monitoring after 14 days of either treatment	- There was a significantly greater increase in fluvoxamine serum concentration in men than in women when the dose doubled (4.6-fold versus 2.4-fold increase)

Table 3: Details of study population, study objectives, methodology and results of trials identified to have a primary objective of exploring age-related differences in pharmacokinetic parameters for anticholinergic medications

Study Author & Study Design	**Study Population**	**Study Objective**	**Methodology**	**Results**
Winchell et al[77] A series of open-label, three-period, randomized, crossover studies	1. 24 healthy young subjects (mean age: 25.5 years; range: 19-39 years; 16 males and 8 females) 2. 18 healthy subjects (mean age: 28.7 years; range: 22-40 years; 8 males, 10 females) 3. 12 older subjects (mean age: 71.3 years; range: 65-79 years; 6 males, 6 females)	To investigate the pharmacokinetics and bioavailability of cyclobenzaprine, including the effects of age and hepatic insufficiency	1. Subjects received 5 mg orally or 1.25 mg IV cyclobenzaprine 2. Subjects received a single oral dose of 2.5, 5, or 10 mg cyclobenzaprine on Day 1 then every 8 hours from Days 8 through 14 and a final dose on Day 15 3. Subjects received 5 mg cyclobenzaprine orally three times daily for 7 days and a final dose on the 8th day	3. Cyclobenzaprine plasma concentrations after multiple dosing were significantly higher for the older compared to young subjects - After the first dose, plasma concentration profiles were similar in older and young subjects - Mean accumulation ratio was 7.9 for older subjects compared to 4.3 for young subjects, and mean effective $T_{1/2}$ was 33.4 h (range: 20.0-53.4 h) in older subjects compared to 18.4 h (range: 9.3-41.3 h) in young subjects
Malhotra et al[97] Two randomized double-blind placebo-controlled trials	1. 32 healthy males aged 18-45 2. 16 young men, 16 older men and 16 older women	To examine the effect of age, sex and race on the pharmacokinetics, pharmacodynamics and safety profiles of fesoterodine	Subjects received either 8 mg of fesoterodine extended release or matching placebo with blood samples drawn over 36 h after drug administration	Renal clearance was 28% lower in older men and women than younger men



Receptor	Most Common Locations	Functional Response (agonism)	Anticholinergic Side Effect (antagonism)
M1	Cerebral cortex, hippocampus and striatum, autonomic ganglia, gastric and salivary glands, enteric nerves	Increase cognitive function – learning and memory Increase seizure activity	Delirium, sedation, confusion
M2	CNS, heart, smooth muscle, autonomic nerve terminals	Heart – SA node: slowed spontaneous depolarization, hyperpolarization, decrease HR AV node: decrease conduction velocity Atrium: decrease refractory period, decrease contraction Ventricle: slight decrease in contraction	Increased heart rate, arrhythmia
M3	CNS, smooth muscle and glands	Increase contraction (predominantly in bladder smooth muscle) Increase secretion (predominant in salivary glands) Increase tremor	Urinary retention, decreased salivation
M4	CNS forebrain	Inhibition of neurotransmitter release	Delirium, sedation, confusion
M5	Rare – CNS and periphery	Facilitates dopamine release Involved with drug seeking behaviour	Reduced drug seeking

Figure 1: Description of the five muscarinic receptor subtypes, their distribution throughout the body, and effect of agonism or antagonism at each muscarinic receptor subtype.

Appendix 1 PRISMA Style Flow Diagram