CHAPTER 2

LISTERATURE REVIEWS

2.1 Quetiapine

Quetiapine (Seroquel[®]) is in the same family as clozapine and olanzapine, which are classified as atypical antipsychotics (Sachse *et al*., 2005). It belongs to the group of the dibenzothiazepines. The chemical designation is 2-[2-(4-dibenzo[b,f][1,4]thizaepine-11-yl-1-piperazinyl)ethoxy]ethanol. Its molecular formula is $(C_{21}H_{25}N_3O_2S)_2C_4H_4O_4$ (Figure 1) and its molecular weight is 833.11 (Hendrickson, 2006)
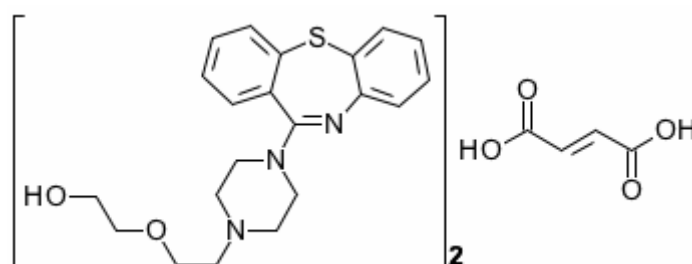

Figure 1. Structural formula of quetiapine (Hendrickson, 2006).

Physical Properties

Powder color	:	white to off-white
Ionization constant	:	pKa1 = 6.83 in phosphate buffer at 22 °C
		pKa2 = 3.32 in formic buffer at 22 °C
Melting point	:	172 °C-174 °C
Solubility	:	very slightly soluble in ether, slightly soluble in water and soluble in 0.1N HCl
Stability	:	15 °C-30 °C

Composition

Quetiapine is available in 5 strengths containing 25, 100, 150, 200 and 300 mg per tablet. The core of the tablet contains the excipients povidone, calcium hydrogen phosphate, microcrystalline cellulose, sodium starch glycolate type A, lactose monohydrate and magnesium stearate. The coating of tablet contains hydroxypropyl methycellulose 2910, polyethylene glycol 400, titanium dioxide, yellow ferric oxide (25-mg, 100-mg and 150-mg tablets) and red ferric oxide (25-mg tablet) (AstraZeneca, 2006).

2.1.1 Mechanism action of quetiapine

Dysfunction of central dopaminergic neurotransmission has been implicated in the pathogenesis of schizophrenia. Increased subcortical dopamine release has also been associated with the pathogenesis of positive symptoms in schizophrenia and may be driven by a prefrontal dopaminergic dysfunction (Heinz, 2002). The positive symptoms are possibly more closely associated with receptor hyperactivity in the mesocaudate, whereas negative symptoms are mostly closely related to dopamine receptor hypofunction in the prefrontal cortex (Crismon *et al*., 2002). Antipsychotic effect is believed to be achieved by inhibition of dopaminergic transmission in the mesolimbic pathway (Flagstad, 2006).

The exact mechanism by which quetiapine exerts its antipsychotic effect is unknown. However, quetiapine is antagonist at multiple neurotransmitter receptors, including 5-HT_{1A}, 5-HT_{2A}, D_1 and D_2, histamine (H_1), alpha-1 and alpha-2 adrenergic receptors (Goren and Levin, 1998; Cutler *et al*., 2002; Moor and Jefferson, 2004). Its antipsychotic action may be due to blocking of dopamine receptors in the mesolimbic pathway because over activity in this location is responsible for positive symptoms. Blockade of the remaining dopamine pathways cause adverse effects rather than a therapeutic benefit. The mesocortical tract is responsible for higher order thinking and executive functions and therefore dopamine hypo-functioning in this area may be responsible for negative symptoms. The nigrostriatal pathway modulates body movement. Quetiapine induces blockade of the dopamine pathway in the tubero-infundibular area of the anterior pituitary leads to hyperprolactinemia.

Blockade of $5HT_2$ receptors in the mesocortical area is another proposed mechanism of antipsychotic action. $5HT_2$ receptor blockade may enhance dopaminergic transmission, thereby relieving negative symptom (Koda-Kimble et al., 2001).

Typical antipsychotics bind more tightly than dopamine itself to the dopamine D_2 receptor, with dissociation constants that are lower than that of dopamine. The newer, atypical antipsychotics such as quetiapine, clozapine and olanzapine all bind more loosely than dopamine to the dopamine D_2 receptor and have dissociation constants higher than that of dopamine. For instance, radioactive haloperidol, chlorpromazine, and raclopride all dissociate very slowly over a 30-minute time span, while radioactive quetiapine and clozapine dissociate rapidly, in less than 60 seconds. Conversely, the occupation of D_2 by clozapine or quetiapine has mostly disappeared after 24 h (Seeman, 2002).

Quetiapine has a higher affinity for $5\text{-}HT_2$ than D_2 receptors. It has the lowest D_2 receptor binding at clinical dose of 300-600 mg/day, D_2 binding range from 0% to 27%. Even at 800 mg/day, only 30% of D_2 receptors are occupied at the same daily doses, whereas 45% to 90% of $5\text{-}HT_{2A}$ receptors are occupied (Crismon et al., 2002). Kapur et al (2000) found that quetiapine leads to transiently high D_2 occupancy of 58% to 64% during the first 2 to 3 h, which falls to minimal levels by 12 h. This suggests that transient occupancy may be sufficient for an antipsychotic effect. Like clozapine, its low level of D_2 occupancy may account for its very low risk of extrapyramidal side effects (EPS) and prolactin elevation. This may also explain why doses of 150-300 mg/day show questionable efficacy (Small et al., 1997) since the dose of quetiapine required to reach a peak occupancy of 60% would be 600-800 mg/day or above. Akadede et al. (2005) suggests that quetiapine improves specific areas of neurocognitive function and suppresses positive and negative symptoms of schizophrenia, without an increase in motor side effects.

Four dopamine pathways in the brain (Figure 2) play a role in the pathophysiology of schizophrenia as well as the therapeutic effects and side effects of antipsychotic agents. Serotonin has importance influences on dopamine, but that influence is quite different in each of the four dopamine pathways. Serotonin inhibits dopamine release from dopaminergic axon terminals in the various dopamine

pathways, but the degree of control differs from one dopamine pathway to another (Stalh, 2000).

Nigrostriatal Dopamine Pathway

The nigrostriatal dopamine pathway extends from the A9 cell group of the substantia nigra to the corpus striatum, which consists of the caudate nucleus, globus pallidus and putamen (Petty, 1999). This pathway as part of the extrapyramidal nervous system, controls movements, and blockade of D_2 receptors in this pathway causes the drug-induced movement disorders EPS and, eventually, tardive dyskinesia. Dopamine deficiency as well as receptor blockade in this pathway can also cause akathisia and dystonia (Stahl, 2003).

Serotonin neurons from the brainstem raphe innervate the dopamine cell bodies in the substantia nigra and also project to the basal ganglia, where serotonin axon terminals are in close proximity to dopamine axon terminals. In both areas, serotonin interacts with postsynaptic 5-HT_{2A} receptors on the dopamine neurons, and this inhibits dopamine release. Thus in the nigrostriatal dopamine pathway, serotonin exerts powerful control over dopamine release because it occurs at two levels. At the level of serotonergic innervation of the substantia nigra, axon terminals arriving from the raphe synapse on cell bodies and dendrites of dopaminergic cells. 5-HT_{2A} antagonism fortunately reverses D_2 antagonism in this pathway. Blocking 5-HT_{2A} receptors should promote dopamine release. When dopamine release is enhanced by quetiapine via blockade of 5-HT_{2A} receptors, this allows the extra dopamine to compete with the quetiapine to reverse the blockade of D_2 receptors. This leads to a reduction or even an absence of EPS and tardive dyskinesia, because there is a reduction of D_2 receptor blockade in this pathway.

Mesolimbic Dopamine Pathway

The mesolimbic pathway, which arises from the A10 cell group lying medial to the substantia nigra, surrounds the inter-peduncular nucleus and innervates the septal nuclei, the amygdala, the olfactory area, and the nucleus accumbens (Petty, 1999). Hyperactivity in the mesolimbic dopamine pathway is thought to cause psychosis and the positive symptoms of schizophrenia such as hallucinations and

delusions. This pathway is also thought to be involved in emotion and sensations of pleasure. Blocking hyperactivity in this pathway should reduce or eliminate positive symptoms (Stahl, 2003).

Mesocortical Dopamine Pathway.

The mesocortical pathway, which also arises from the same A10 cell group, projects to the frontal, cingulate, and entorhinal cortices (Petty, 1999). This pathway is thought to control cognitive function, and dopamine deficiency in this pathway may be responsible for the negative and cognitive symptoms of schizophrenia and therefore, dopamine receptor blockade in this pathway would theoretically lead to a worsening of negative and cognitive symptoms. In other words, an agent would have to decrease dopamine in the mesolimbic pathway to alleviate positive symptoms but increase it in the mesocortical pathway to treat negative and cognitive symptoms (Stahl, 2003). Role of 5-HT_{1A} receptors activation may modulate dopaminergic neurotransmission in the prefrontal cortex. It is critically involved in the regulation of dopamine release, which is involved in key cognitive function and possibly also in mood regulation (Moller, 2005).

Tubero-infundibular Dopamine Pathway

The tubero-infundibular tract, which is a system of short axons running along the base of hypothalamus, releases dopamine into the portal veins of the pituitary gland (Petty, 1999). Normal function of the tubero-infundibular dopamine pathway inhibits prolactin release. If normal function of this pathway is disrupted, for example, by D_2-blocking drugs, hyperprolactinemia can occur, with side effects such as galactorrhea, amenorrhea, and sexual dysfunction (Stahl, 2003). When D_2 receptors are blocked by a conventional antipsychotic, dopamine can no longer inhibit prolactin release, so prolactin levels rise. However, in the case of atypical antipsychotic quetiapine, there is simultaneous inhibition of 5-HT_{2A} receptors, so serotonin can no longer stimulate prolactin release.

D_2 receptor blockade may have a beneficial outcome in one pathway but it may cause other problems in other pathways (Table 1).

Table 1. Dopaminergic tracts and effects of dopamine antagonists (Crismon *et al*, 2002)

| Dopamine pathway	Origin	Innervation	Function	Dopamine antagonist effect
Nigrostriatal	Substantia nigra (A9)	Caudate nucleus, putamen	Extrapyramidal system, movement	Movement disorders
Mesolimbic	Midbrain ventral tegmentum (A10)	Limbic system (amygdala, olfactory, tubercle, septal nuclei), cingulate gyrus	Memory, stimulus processing, motivational behaviors	Relief of psychosis
Mesocortical	Midbrain ventral tegmentum (A10)	Frontal and pre-frontal lobe cortex	Cognition, communication, social function, response to stress	Relief of psychosis
Tubero-infundibular	Hypothala-mus	Pituitary gland	Regulates hormone and prolactin release	Increase prolactin but decrease other hormones
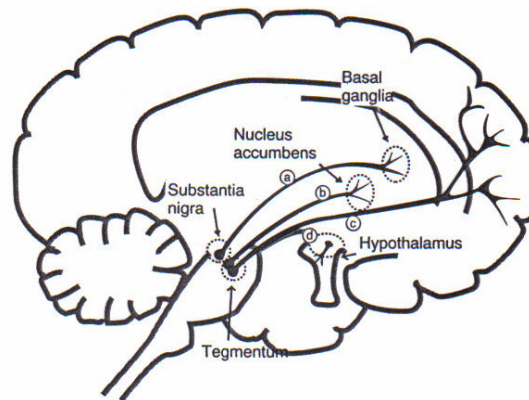
Figure 2. Four dopamine pathways in the brain: (a) nigrostrital dopamine pathway; (b) mesolimbic dopamine pathway; (c) mesocortical dopamine pathway; and (d) tubero-infundibular dopamine pathway (Stahl, 2000).
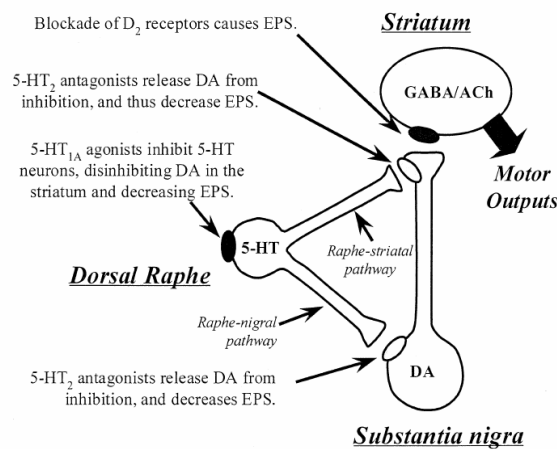

Figure 3. This schematic diagram illustration is a simplification of the varied and complex interactions of dopamine and serotonin (Lieberman *et al*., 1998).

Serotonergic neurons in the median and dorsal raphe nuclei innervate DA neurons (Figure 3) in the substantia nigra (A9) and the ventral tegmental area (A10). In addition to innervation of A9 and A10 neurons, 5-HT neurons project to terminal fields of DA neurons, including the caudate putamen, nucleus accumbens, medial prefrontal cortex and amygdala. Serotonin regulates dopamine release: the presence of serotonin in some dopamine pathways such as the nigrostriatal pathway,

inhibits the release of dopamine, whereas in the mesolimbic dopamine pathway, serotonin has little or no effect. When 5-HT_{2A} receptors are blocked, dopamine is released in the nigrostriatal pathway but is not released in the mesolimbic pathway. The naturally occurring dopamine is then "dis-inhibited" and filled D_2 receptors, preventing blockade by quetiapine, thus motor side effects are reduced. However, dis-inhibition in the nigrostriatal pathway does not affect the blockade of D_2 binding in the mesolimbic pathway (Stahl, 2003).

2.1.2 Pharmacokinetics of quetiapine

Absorption: Quetiapine is rapidly absorbed from GI tract. The bioavailability of quetiapine is marginally affected by administration with food, with C_{max} and AUC values increased by 25% and 15% respectively. Peak plasma concentrations are reached in 1 to 2 h (Sadock, 2001). Jaskiw *et al*. (2004) assess the pharmacokinetics of quetiapine in 12 elderly patients with selected psychotic disorders. Under steady-state conditions, they found that quetiapine is rapidly absorbed after oral administration, with T_{max} ranging from 0.5 to 3 h at the 100mg dose (day 15) and from 1 to 3 h at the 250 mg dose (day 23). These results indicate that the pharmacokinetics of quetiapine is linear in the elderly population. Quetiapine should be start at lower doses and titrated at a relatively slower rate in patients $\geq$ 65 y. When study in Chinese suffering from schizophrenia who were given quetiapine twice daily, after the dose reached 200 mg twice daily, quetiapine is rapidly absorbed with a mean T_{max} about 2 h (Li *et al*., 2004).

The absolute bioavailability is unknown, but the relative bioavailability from orally administered tablets compared with a solution is nearly complete (DeVane and Nemeroff, 2001). Therefore the tablet formulation is 100% bioavailable relative to solution.

Distribution: Quetiapine is widely distributed throughout the body with an apparent volume of distribution of 10±4 L/kg (AstraZeneca, 2006). After the dose reaches 200 mg twice daily, the main V/F is 672 L (Li *et al*., 2004), which indicates quetiapine is widely distributed throughout the body. It is 83% bound to plasma proteins at therapeutic concentrations (AstraZeneca, 2006). Quetiapine passes

the human placenta but that the blood-placental barrier partially limits the transplacental transfer of quetiapine (Rahi *et al*., 2007).

Metabolism: Principal elimination pathways of quetiapine (Figure 4) include sulfoxidation, oxidation of the terminal alcohol to the corresponding carboxylic acid, hydroxylation of dibenzothiazepine ring, O-dealkylation, N-dealkylation and phase II conjugation (Wrighton and Thummel *et al*., 2000). The principal human plasma metabolites are sulfoxide and the parent acid metabolite, neither of which are pharmacologically active (AstraZeneca, 2006). Quetiapine is mainly metabolized in the liver and hepatic metabolism accounts for the formation of at least 11 metabolites (Mandrioli *et al*., 2002). Of all the metabolites, only 7-hydroxy and N-dealkylation metabolites are considered to be active (Goren and Levin, 1998). DeVane and Nemeroff *et al*. (2001) reported that metabolism of quetiapine is mainly catalyzed by CYP3A4. CYP3A4 has been demonstrated to be responsible for sulfoxidation, N- and O-dealkylation of quetiapine, and partially responsible for 7-hydroxylation. CYP2D6 plays a minor role in the metabolism of quetiapine as CYP3A4 contributes for 89% of the overall metabolism (Hasselstrom and Linnet, 2006) and may also play a role in the 7-hydroxylation pathway (Grimm *et al*., 1997). *In vivo*, quetiapine sulfoxide (QTP-SF) is the major inactive metabolite. 7-hydroxy-quetiapine (QTP-OH) and 7-hydroxy-N-dealkyl-quetiapine (QTP-ND) are active metabolites (Gefvert *et al*., 1998). Quetiapine and its metabolites have little inhibitory effect on the *in vivo* metabolism mediated by CYP1A2, 2C9, 2C19, 2D6 or 3A4.
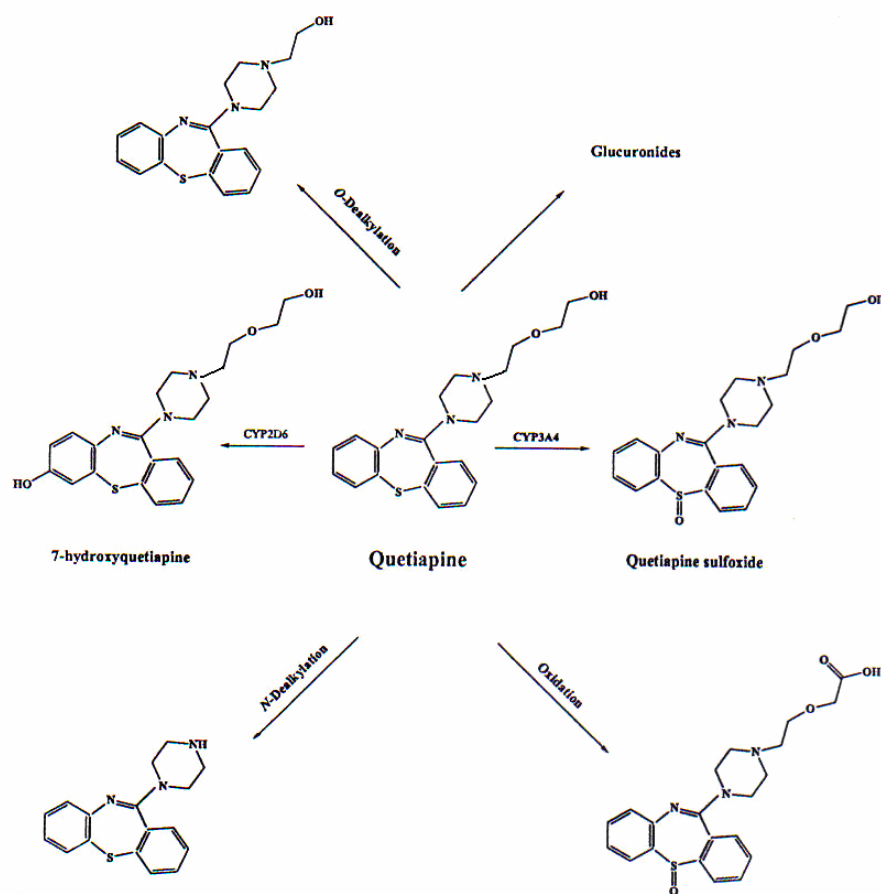
Figure 4. Metabolic profile of quetiapine (Wrighton and Thummel, 2000)

Excretion of quetiapine: Quetiapine is mainly metabolized by liver with a mean terminal half-life of about 6 h. Less than 1% of the administered oral dose is excreted unchanged in urine and feces. Approximately 73% and 21% of the dose are quetiapine-related material excreted in the urine and feces, respectively (Schatzberg and Nemeroff, 2001)

2.1.3 Dosage and administration

Quetiapine is initiated at a dosage of 25 mg twice a day and then increased on day 2 to 50 mg twice a day, on day 3 to 100 mg twice a day and on day 4 to 100 mg in the morning and 200 mg in the evening. The optimal dosage for most patients appears to range between 400-600 mg/day, although the drug is safe and

efficacious for some patients within a dose range of 150-750 mg (Cutler *et al.*, 2002). A slower titration and lower daily doses may be warranted for patients with hepatic disease and for elderly patients. Because of its relatively short half-life, quetiapine is usually administered twice daily (Hales and Yudofsky, 2004). Most people receiving maximum benefit at 300-500 mg/day (Sadock, 2001).

2.1.4 Therapeutic efficacy

Schizophrenia: The efficacy and safety of quetiapine were tested in 109 schizophrenic patients in a multi-center, randomized, double-blind, placebo-controlled, parallel group trial (Borison, 1996). Subjects randomized to quetiapine initially received 25 mg three times per day for 1-2 days. Thereafter, the dose was titrated upward to a maximum daily dose of 750 mg. By day 21 quetiapine was clinically and statistically superior to placebo in moderating negative symptoms.

Small *et al.* (1997) studied in 286 subjects to evaluate quetiapine at high doses (> 250 mg but ≤ 750 mg), low doses (≤ 250 mg) or placebo. Only the higher doses are related to significantly greater improvement when compared to placebo, suggesting that the effective dose is greater than 250 mg.

Arvanitis and Miller (1997) reported a multiple fixed-doses, placebo-controlled, double-blind study of quetiapine in comparison with haloperidol and placebo in acutely exacerbated patients with chronic schizophrenia. Quetiapine was administered in five doses: 75, 150, 300, 600 and 750 mg/day. Haloperidol was given at 12 mg/day. The study design had slightly more than 50 patients in each group. The 75 mg dose of quetiapine is clearly less efficacious than the higher doses. Dose of 150 to 750 mg/day are superior to placebo and comparable with haloperidol in reducing positive symptoms and the dose of 300 mg/day is superior to placebo and comparable with haloperidol for negative symptoms.

Copolov *et al.* (2000) studied in 448 acutely psychotic patients comparing the efficacy of quetiapine (mean dose 455 mg/day) and haloperidol (mean dose 8 mg/day). This study found similar efficacy for the two agents.

Emsley *et al.* (2000) compared 600 mg/day of quetiapine to 20 mg/day of haloperidol in patients only partially responsive or non-responsive to a trial of

fluphenazine (20 mg/day). There is a non-significant trend toward an advantage for quetiapine.

Mullen (2001) compared quetiapine (mean dose 254 mg/day) to risperidone (mean dose 4.4 mg/day) in an open-label study in 728 outpatients who were having their medications changed. The result indicates that the two drugs are similar in efficacy and tolerability.

Mania: Quetiapine has been found to be efficacious in the treatment of acute mania, as mono-therapy (Bowden *et al*., 2005; McIntyre *et al*., 2005) or in combination with other mood stabilizers (Sachs *et al*., 2004), as well as mono-therapy in bipolar depression (Calabrese *et al*., 2005). The superior efficacy of quetiapine in combination with lithium or divalproex compared with lithium or divalproex alone in acute mania has been established in a large study (Sachs *et al*., 2004). The usual quetiapine dosage used in previous mania studies was up to 800 mg/day leading to response rate between 42.6% to 55.7% in the quetiapine groups at day 21 (Bowden *et al*., 2005; McIntyre *et al*., 2005; Sachs *et al*., 2004).

Bipolar: Dando and Keating (2005) reported that quetiapine shows efficacy in the treatment of acute mania and depression associated with bipolar disorder. Quetiapine is well tolerated and effective in reducing manic symptoms in adult and adolescent patients with acute bipolar mania and approved for use in adults for this indication.

Pini *et al*. (2006) evaluated the efficacy and tolerability of quetiapine in the acute and maintenance phase of bipolar disorder. Quetiapine has been found to be effective as adjunctive therapy in combination with lithium or valproate, significantly superior to placebo, and equal to lithium or haloperidol as mono-therapy.

Buckley *et al*. (2007) evaluated the effects of quetiapine on agitation and aggression in 407 patients with bipolar I mania randomized to quetiapine mono-therapy (200-800 mg/day) or placebo for 12 weeks, and 402 patients were randomized to quetiapine (200-800 mg/day) or placebo in combination with lithium or divalproex for 3 or 6 weeks. Measurements of agitation included the Positive and Negative Syndrome Scale (PANSS) Activation subscale, PANSS Supplemental Aggression Risk subscale scores, and Young Mania Rating Scale (YMRS) items relevant to agitation. The results found that the reduction in PANSS is significantly greater with

quetiapine mono-therapy than placebo. They suggested that quetiapine is an effective and appropriate treatment choice in managing agitation and aggression associated with bipolar mania. In the same year, Khazaal *et al*. (2007) study confirms the quetiapine efficiency and tolerability in the treatment of the affective episodes in bipolar patients.

Depression: Quetiapine is an effective augmenting agent in the treatment of resistant depression (Doree *et al*., 2007). This study compared 20 major depression patients who had failed to respond to treatment with an antidepressant. Patients were randomized to receive either lithium (600 mg/day) or quetiapine (400 mg/day). The results found that quetiapine group shows greater improvement than the lithium group.

Dementia: Zhong *et al*. (2007) reported that quetiapine 200 mg/day is effective and well tolerated for treating agitation associated with dementia.

Onor *et al*. (2006) evaluated the efficacy and tolerability of quetiapine in a group of patients with a diagnosis of dementia and concomitant psychotic disorders. Tolerability was assessed by the incidence of clinically evident side effects. The results show that quetiapine is effective in reducing behavioral symptoms, delirium and hallucinations, aggressiveness, and sleep disturbances. Quetiapine tolerability has proved to be satisfactory. The only side effect of clinical significance is orthostatic hypotension, which is, however, partially preventable by a slower drug titration.

2.1.5 Efficacy of short-term and long-term treatment

Quetiapine has established efficacy and good tolerability in the short-term and long-term treatment in schizophrenia. An analysis of open-label extension studies found that patients continue to improve when treated long term with quetiapine (Kasper *et al*., 2004). Its beneficial effects have been shown to persist for at least 52 weeks (Palmer, 2005).

Judit (2005) study in open label 35 hospitalised patients with psychosis, who received quetiapine at doses up to 1,600 mg/day in a 4-week acute phase, were followed for up to 14 months as outpatients. The results at the end of the 4-week hospitalization period showed that overall 94.3% of patients experience

improvements in symptoms, with 37.1% very much improve and 20% minimally improve. Among the 12 patients receiving > 800 mg/day, 83% are very much improved and no increase in extrapyramidal symptoms or other adverse events is observed at dose above 800 mg/day. These results indicate that short-term quetiapine therapy at dose up to 1,600 mg/day with maintenance doses up to 1,000 mg/day may be an effective and well tolerated treatment for patients with psychosis.

Glick and Marder (2005) compared 1-year outcomes in stabilized patients with schizophrenia randomly assigned to either oral quetiapine or haloperidol decanoate. Treatment was open labeled but raters were blind. Relapse rate is similar for both agents, with an advantage for quetiapine over haloperidol for extrapyramidal and negative symptoms response.

Tariot *et al*. (2000) performed a long-term (52-week) study in 184 elderly patients aged $\geq$ 65 years with psychotic disorders. They found that quetiapine is effective, well tolerated, and safe, and offers clinical benefit in elderly.

2.1.6 Side effects

The most common side effects of quetiapine, compared with placebo are somnolence and dizziness. Quetiapine can produce orthostatic hypotension in about 7% of patients and 1% may experience frank syncope following rapid titration of the dose (Schatzberg *et al*., 2005).

Data on file (AstraZeneca 2006) reported the side effects of quetiapine as follows:

Somnolence: Somnolence is one of the most common side effects of quetiapine, may occur during the first two weeks of treatment, which generally resolves with continued administration of quetiapine. Somnolence is dose dependent and patients often become tolerant to this side effect over time (Hales and Yudofsky, 2004). During the clinical trials, 18% of the quetiapine patients compared to 11% of placebo patients experienced this side effect.

EPS: EPS are not common with quetiapine. As with olanzapine, there have been rare reports of tardive dyskinesia and no clear estimates of the frequency of tardive dyskinesia with quetiapine are available (Schatzberg *et al*., 2005).

Weight gain: Weight gain associated with quetiapine seems to be less than that seen with olanzapine and clozapine but more than that seen with ziprasidone and risperidone (Schatzberg *et al*., 2005). During acute therapy in placebo-controlled schizophrenia clinical trials, mean weight gain in patients taking quetiapine is 2.3 kg when compared to a mean weight gain of 0.1 kg in patients taking placebo. Nasrallah's report (2003) showed that 2,216 patients who participated in a long-term (12 months) trial with quetiapine treatment, gain a small mean weight increase of 2.08 kg.

Seizures: There have been occasional reports of seizures in patients administered quetiapine, although the frequency is no greater than that observed in patients administered placebo in controlled clinical trials.

Priapism: There have been very rare reports of priapism in patients administered quetiapine. Pais and Ayvazian (2001) reported a first case of priapism occurring after an overdose of quetiapine. This case was a 45-year-old man with a history of depression and bipolar disorder. He ingested 27 quetiapine 25-mg tablets.

Prolactin level: Quetiapine has negligible effect on the elevation of prolactin. In all of the large trials of quetiapine, prolactin levels have been reported to decrease from baseline to endpoint during quetiapine treatment and no differences are noted between quetiapine and placebo (Conley and Kelly, 2004). Similarly, Fleischhacker *et al*. (1996) found that substitution with quetiapine is associated with a reduction in mean serum prolactin levels, whereas haloperidol is associated with an increased mean prolactin level. The mean level in the haloperidol group is significantly higher than that in the quetiapine group ($P < 0.01$). Stevens *et al*. (2005) studied in a cross-sectional retrospective medical chart review of 70 male youths, 50 males treated with risperidone and 20 males treated with quetiapine. Serum prolactin levels were drawn according to a protocol, after at least 6 weeks of treatment. They reported that prolactin is above the upper limit of normal for 68% of the patients on risperidone and 20% of the patients on quetiapine. Both risperidone and quetiapine produce dose-related increases in serum prolactin levels. No correlation has been found between duration of treatment and prolactin levels.

Impotence: Abnormal ejaculation and amenorrhea have been reported in pivotal trials to occur in less than 0.1% of patients. In over 2,000 patients treated with quetiapine, menstrual change occurs in less than 1% (Conley and Kelly, 2004).

Ocular change: The development of cataracts has been observed in association with quetiapine treatment in preclinical studies of dogs, but a causal relation has not been established in humans. Post-marketing experience has not detected an increase in incidence of cataracts with quetiapine compared with other antipsychotics, however, cataracts are in general more common in schizophrenia compared with the general population (Hales and Yudofsky, 2004).

Cardiovascular effects: As predicted with alpha-1 antagonism, quetiapine may induce orthostatic hypotension and concomitant symptoms of dizziness, tachycardia and syncope, especially during the initial dose-titration period. Syncope was reported in 1% of the patients treated with quetiapine. This risk is minimized by limiting the initial dose to 25 mg twice daily (bid).

Hepatic effects: Asymptomatic, transient and reversible elevation in serum transaminases (primarily ALT) has been reported in patients taking quetiapine in premarketing evaluation. In the clinical trials the proportions of patients with transaminase elevations of >3 times the upper limit were 6% vs 1% for placebo.

McIntyre *et al*. (2005) conducted a randomized, 12 weeks double-blind treatment with quetiapine, placebo or haloperidol in 302 bipolar patients. The common adverse events with quetiapine are somnolence (12.7%), insomnia (19.6%) and EPS-related. Similarly, King *et al*. (1998) studied in 618 patients comparing bid and three time daily (tid) dosage regimens of quetiapine in a 6-week, double-blind, randomized, multi-center, parallel-group study. The results found that quetiapine is generally well tolerated, with no difference in the tolerability profile observable between the 225 mg bid and 150 mg tid groups. Most classes of adverse events occur in less than 10% of the patients (Table 2), and the majority of these events are apparently independent of the dose prescribed such as insomnia, anxiety and agitation.

Table 2. Adverse events in three treatment groups (adapted from King *et al*., 1998)

Adverse event	25 mg bid (n=200)		150 mg tid (n=209)		225 mg bid (n=209)	
	Number of patients	*%*	Number of patients	*%*	Number of patients	*%*
Somnolence	17	8	29	14	27	13
Insomnia	19	9	16	8	20	10
Dry mouth	6	3	10	5	16	8
Dizziness	4	2	12	6	11	6
Asthenia	3	1	7	3	9	5
Postural hypotension	10	5	12	6	8	4
Anxiety	8	4	13	6	8	4
Agitation	11	5	8	4	6	3
Headache	10	5	10	5	5	3

2.1.7 Drug interactions

Quetiapine is primarily metabolized by CYP enzymes. When co-administered with inducers or inhibitors (psychotropic or non-psychotropic medications or substances) of CYP enzymes, antipsychotic plasma levels may be reduced or increased, respectively, as a result of drug interactions. This can result in a reduced effectiveness of the antipsychotic, or an increased risk of adverse events, respectively.

Any drugs that are CYP3A4 inhibitors co-administered with quetiapine may lead to increased quetiapine plasma levels. Protease inhibitors (ritonavir, indinavir, and atazanavir) are potent CYP3A4 inhibitors, as are antifungal agents

(ketaconazole), macrolides (troleandomycin, erythromycin), and nefazadone. (Conley and Kelly , 2007).

Ketoconazole: In a multiple dose trial in healthy volunteers to assess the pharmacokinetics of quetiapine given before and during treatment with ketoconazole showed that it increases mean quetiapine C_{max} and AUC of 235% and 522%, respectively, with corresponding decrease in mean oral clearance of 84%. The mean half-life of quetiapine increases from 2.6 to 6.8 h, but the mean T_{max} is unchanged (Goren and Lavin, 1998). These results are in line with that of Grimm *et al*. (2005) who found that ketoconazole increases mean quetiapine plasma by 3.35 folds and decreases its clearance by 84%.

Erythromycin: Li *et al*. (2005) studied the effects of erythromycin on the metabolism of quetiapine in Chinese suffering from schizophrenia, 19 patients received multiple doses of quetiapine 200 mg twice daily with or without co-administered erythromycin (500 mg, three times daily). They found that erythromycin increases quetiapine C_{max}, AUC, $T_{1/2}$ by 68%, 129%, and 92%, respectively. It decreases quetiapine clearance 52%. Erythromycin has a noticeable effect on the metabolism of quetiapine. When quetiapine is co-administered with CYP3A4 inhibitors such as erythromycin, the dosing regimen should be modified according to quetiapine serum concentrations.

Cimetidine: Strakowski *et al*. (2002) studied the effects of multiple doses of cimetidine (CYP 3A4 inhibitor) on the steady-state pharmacokinetics of quetiapine in 13 patients with selected psychotic disorders. Quetiapine was maintained at 150 mg three times daily and cimetidine 400 mg. They found a slight increase in quetiapine plasma levels and a reduction in oral clearance after cimetidine co-administration.

Fluoxetine and Imipramine: Potkin *et al*. (2002) investigated 26 patients with schizophrenia in a multi-center, two-period, multiple-dose, open-label randomized trial. Patients were treated with 300 mg twice daily dose of quetiapine for at least 7 days and received fluoxetine 60 mg or imipramine 75 mg for 8 days. They found that co-administration of quetiapine with fluoxetine leads to an increase in $AUC_{0-12\ h}$ of 12% and C_{max} of 26%; these increases are deemed statistically

significant, although not clinically significant, and result in no adverse events. On the otherhand, imipramine does not affect the pharmacokinetics of quetiapine.

Divalproex: Co-administration of quetiapine (150 mg, bid) and divalproex (500 mg bid) increases the mean maximum plasma concentration of quetiapine by 17% without changing the mean oral clearance (AstraZeneca, 2006)

Any potent CYP3A4 inducer that is co-administered with quetiapine may result in an increased dose of quetiapine being required to achieve the original desired therapeutic effect.

Phenytoin: Phenytoin, a potent CYP3A4 inducer, markedly decreases mean plasma levels of quetiapine and, consequently, decreases its therapeutic benefit. Wong *et al.* (2001) studied the effects of concomitant phenytoin administration on the steady-state pharmacokinetics of quetiapine. The quetiapine geometric mean AUC_{0-8h}, C_{max}, and C_{min} are reduced to 19%, 27%, and 12% of their former values, respectively, after the administration of phenytoin. Quetiapine CL/f increased more than 5 folds after phenytoin co-administration. This study demonstrates that the potent CYP450 inducer, phenytoin causes 5-fold increase in the clearance of quetiapine and suggests that dosage adjustment of quetiapine may be necessary when the two drugs are given concurrently.

Thioridazine: It significantly increases the oral clearance of quetiapine and, consequently, doses of quetiapine may need to be increased during co-administration with thioridazine to achieve the necessary control of psychotic symptoms (Potkin *et al.*, 2002).

Carbamazepine: This drug decreases quetiapine plasma by 80% and increases its clearance 7.5 folds (Grimm *et al.*, 2005).

2.1.8 Overdose

Kurth and Maguire (2004) studied a case of 14-year-old boy with a history of major depressive disorder ingested 1,900 mg of quetiapine. This report presents a higher serum levels quetiapine and QTc prolongation after 1 and 1.5 h ingestion.

In clinical trials, experience with quetiapine in overdose is limited, estimated doses of up to 20 g of quetiapine have been taken, no fatalities have been

reported and patients recover without sequelae. In post-marketing experience, there have been cases of coma and death in patients taking a quetiapine overdose. The lowest reported dose associated with coma has been in patients who took 5 g and had a full recovery within 3 days. The lowest reported dose associated with a death was in patients who took 10.8 g.

Parker and McIntyre (2005) determined the toxicity of quetiapine in 21 postmortem examined cases. Specimens analyzed were peripheral blood, central blood, liver, vitreous humor and gastric contents. Finding from this study suggest that therapeutic postmortem quetiapine concentration may be less than 1 mg/L in both peripheral and central blood, less than 0.5 mg/L in vitreous and less than 5 mg/kg in liver. Quetiapine concentrations indicative of toxicity have been estimated at greater than 1 mg/L in peripheral and central blood, greater than 0.5 mg/L in vitreous and greater than 5 mg/kg in the liver.

There is currently little information available on quetiapine overdose in the pediatric population. Catalano et al. (2002) presented a case of 15-year-old girl who ingested 1,250 mg of quetiapine in a suicide attempt. She developed multiple symptoms including tachycardia, agitation, hypotension and unconsciousness.

Hunfeld *et al*. (2006) studied 21 intoxicated cases with quetiapine. They found that the ingested doses ranged from 1,200-18,000 mg, the blood concentrations ranged from 1.1-8.8 mg/L with a lag time of 1-26.2 h. The most frequent findings are somnolence and tachycardia. Severity of intoxication is not associated with a higher amount of quetiapine intake. No fatalities occur.

There is no specific antidote to quetiapine. In cases of severe intoxication, the possibility of multiple drugs involvement should be considered, and intensive care procedures are recommended, including establishing and maintaining a patent airway, ensuring adequate oxygenation and ventilation, monitoring and support of the cardiovascular system (AstraZeneca, 2006).

2.1.9 Special patient populations (Cutler *et al*., 2002)

Adolescents: Although there have been no randomized, double-blind studies of quetiapine in children, results of a pilot study in adolescents (aged 12.3-15.9 years) suggest that the dose requirements and clinical responses to quetiapine in

this population are not significantly different from those in adult patients with psychotic disorders.

The elderly: Like other antipsychotic agents, quetiapine should be used with caution in elderly patients, particularly during the initiation of therapy. Dosing should begin at 25 mg/d, increasing by 25 mg/d until an effective dose is reached. Because of the reduced clearance of quetiapine in the elderly, the optimal dose is likely to be lower in this population than in younger patients. This is illustrated by the results of an open-label trial in 18 elderly patients? in which the median dose of quetiapine is 138 mg/d. Consequently, the recommended initial target dose in elderly patients is 100 mg/d.

Renal and hepatic impairment: Dose adjustment of quetiapine is not required in patients with renal impairment. However, because quetiapine is metabolized in the liver, slower dose titration may be desirable in patients with hepatic impairment. Also, depending on individual clinical response and tolerance, the daily therapeutic dose may be lower in patients with hepatic impairment. In these patients, therapy should be started at 25 mg/d and increased by 25 to 50 mg/d to an appropriate dose.

Pregnancy: Quetiapine is a Pregnancy Category C drug and should be used during pregnancy only if the potential benefits outweigh the potential risk to the fetus. Quetiapine has been found in the breast milk of animals administered the drug and women receiving quetiapine should not breast-feed.

Mood and affective disorders: Clinicians are increasingly using atypical antipsychotic agents in patients with mood and affective disorders, and quetiapine has shown potential benefit in this population. Patients with acute psychotic mania appear to require and tolerate higher doses of quetiapine and these antipsychotic medications than patients with stable depression or bipolar.

2.2 Cytochrome P450 (CYP450)

Drug metabolism refers to the processes by which drugs are biochemically modified to facilitate their degradation and subsequent removal from the body. Drug metabolism is normally divided into two phases, phase I and phase II. The reactions of phase I are thought to act as a preparation of the drug for the phase II reaction.

Phase I (biotransformation reactions) usually occurs in the first step and introduces or presents a functional group on the drug molecule. Phase I metabolism includes oxidation, reduction, hydrolysis and hydration reaction. In most cases, the final product contains a chemically reactive functional group, such as -OH, -NH$_2$, -SH, -COOH, etc. The main function of phase I is to prepare the compound for phase II

Phase II (conjugation reactions) involves coupling the drug to endogenous substances, such as glucuronic acid, glycine, glutathione or glutamine (Prior *et al*., 1999), is usually the true detoxification of drugs and yields products that are generally water-soluble and easily excreted. The major conjugation reactions include glucuronidation, sulphation, acetylation, methylation, amino acid conjugation and glutathione conjugation. Glucuronidation and sulphate conjugations are very common phase II reactions that result in water-soluble metabolites rapidly excreted in bile and/or urine.

Studies to date indicate that the CYP3A is the dominant oxidative enzyme in human drug metabolism. The activity of this enzyme system requires both a reducing agent (NADPH) and molecular oxygen. In a typical reaction, one molecule of oxygen is consumed (reduced) per substrate molecule, with one oxygen atom appearing in the product and the other in the form of water. Two enzymes are important in this process:

1). NADPH-CYPP450 reductase**.** One mole of this enzyme (molecular weight of 80,000 d) contains one mole each of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Because cytochrome c can serve as an electron acceptor, the enzyme is often referred to as NADPH-cytochrome c reductase.

2). CYP450**.** The name CYP450 is derived from the spectral properties of this hemoprotein. In its reduced (ferrous) form, it binds carbon monoxide to give a ferrocarbonyl adduct that absorbs maximally in the visible region of the electromagnetic spectrum at 450 nm. Over half of the heme synthesized in the liver is committed to hepatic CYP450 formation. The relative abundance in liver of CYP450, as compared to that of the reductase, makes the reductase the rate-limiting step in hepatic drug oxidations.

Microsomal drug oxidations require CYP450, CYP450 reductase, NADPH, and molecular oxygen (Figure 5). The cycle involves four steps (Katzung, 2001):

1. Oxidized (Fe3+) CYP450 combines with a drug substrate to form a binary complex.

2. NADPH donates an electron to the CYP450 reductase, which in turn reduces the oxidized CYP450-drug complex.

3. A second electron is introduced from NADPH via the same CYP450 reductase, which serves to reduce molecular oxygen and form an "activated oxygen" CYP450-substrate complex.

4. This complex in turn transfers "activated" oxygen to the drug substrate to form the oxidized product. The potent oxidizing properties of this activated oxygen permit oxidation of a large number of substrates.
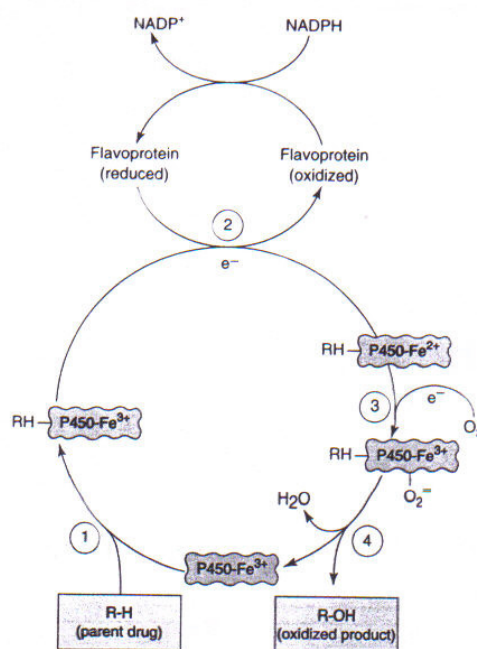
Figure 5. CYP450 cycle in drug oxidations. R-H: parent drug; R-OH: oxidized metabolite; e⁻: electron. (Katzung, 2001).

Mammalian CYP450s are a super-family of heme-containing enzymes that are able to metabolize a wide variety of compounds that act as regulators (e.g., steroids, prostaglandins, thromboxanes, fatty acid derivatives and derivatives of retinoic acid). Another important function of CYP450s is their ability to catalyze the oxidation of xenobiotics such as drugs and environmental pollutants (Otyepka *et al*., 2007). The CYP450 system constitutes a super-family of isoenzymes, located in the membranes of the smooth endoplasmic reticulum in the liver and in many extra-hepatic tissues that mediate oxidative reactions of most drugs and xenobiotics, as well as many endogenous compounds. The multiple CYP enzymes are subdivided into families, subfamilies and isoenzymes according to a nomenclature system based on amino acid sequence homology. The CYP3 family consists of CYP3A4, 3A5, 3A7, and 3A43 (Burton et al., 2006) and two pseudogenes; *3A5P1, 3A5P2* (Daneilson, 2003). On the basis of the concentrations of individual P450 enzymes in human liver microsomes, CYP3A represents 30% of total hepatic P450 content; CYP2C represents 18%; CYP1A2, 13%; CYP2E1, 7%; CYP2A6, 4%; CYP2D6, 1.5%; and CYP2B6, 0.2% (Kashuba *et al*., 2006). CYP3A4 and CYP3A5 are the most abundantly

expressed P450 enzymes in the human liver and gastrointestinal tract, and are known to metabolize more than 120 frequently prescribed drugs and endogenous substrates such as steroids and bile acids. CYP3A5 is much more commonly associated with extra-hepatic tissues including lungs, colon, kidney, esophagus, and anterior pituitary (Nebert, 2002). A single member of the CYP3 family, CYP3A4, is arguably the single most important drug-metabolizing CYP450 enzyme in humans. It is the designation of the cytochrome in family 3, subfamily A, gene product 4 (Prior, 1999). Estimates suggest that CYP3A forms participate in the metabolism of more than 50% of all drugs for which the P450s responsible for their metabolism are known (Wrighton and Thummel, 2000). It accounts for approximately 30-40% of total liver and intestinal CYP content and is responsible for the metabolic transformation of 50-70% of commonly used pharmaceutical drugs (Yengi et al., 2006). CYP3A5 accounts for 5-50% of total CYP3A abundance and is present in appreciable amount in about 25% of the adult population. CYP3A7 is the primary fetal enzyme and is rarely detected in adults. CYP3A43 is detectable in significant amounts in the prostate and testis. Both CYP3A7 and 3A43 appear to play a minor role in drug metabolism in adult population (Zhou et al., 2004). CYP3A4 is known to metabolize a large variety of compounds varying in molecular weight from lidocaine (M.W. = 234) to cyclosporine (M.W. = 1203) (Zhou et al., 2004)

CYP450s are polymorphic: there exist ethnic difference in hepatic enzymes that influence the pharmacokinetics of drugs. Approximately 5% to 10% of Caucasians are poor metabolizers via CYP2D6, while approximately 20% of Japanese and Chinese are poor metabolizers via the CYP 2C19 (Sharif, 2003). Poor metabolism will increase the bioavailability of some drugs, increasing their likelihood of side effects.

2.2.1 Enzyme induction

Enzyme induction is less frequently encountered in clinical practice than enzyme inhibition. It can occur by changing in the rate of enzyme synthesis or the rate of enzyme degradation. Increased levels of enzyme in an eliminating organ, such as the liver, generally results in an increase in the intrinsic metabolic clearance, increased excretion and reduced area under the concentration (AUC)-time profile

(Wrighton and Thummel, 2000). Induction results in an acceleration of metabolism and usually in a decrease in the pharmacologic action of the inducer and also of co-administered drug. This increased enzyme activity is sometimes accompanied by hypertrophy of the endoplasmic reticulum. There is a rise in CYP450 content and increased CYP450 reductase activity. Enzyme induction is a dose-dependent process.

Induction of cytochrome activity occurs at the level of gene transcription. Probably the most important drugs that act as inducers are ethanol, rifampin (a drug used to treat tuberculosis), the barbiturates (e.g., phenobarbital), and two antiepileptic drugs - phenytoin and carbamazepine. The inducers stimulate the transcription of genes encoding CYP450 enzyme, and results in increased messenger RNA and protein synthesis (Brenner, 2000)

Barbiturates, glucocorticoids, polycyclic aromatic hydrocarbons, alcohol and isoniazid are examples of agents that cause synthesis of new P450 enzyme molecules. The time course of enzyme induction onset and offset is closely related to the plasma concentration of the inducer, as well as the half-life of enzyme production and degradation (Gram, 1997). The time-course of induction varies with different inducing agents. For example, rifampicin can produce noticeable changes in the activity of the hepatic drug metabolizing enzymes within 48 h. The half time of shift from one steady state to another is theoretically, a function of enzyme turnover and the half-life of the enzyme. In clinical practice, most inducing agents administered in therapeutic doses will produce maximum effect within 14 days (Na-Bangchang and Wernsdorfer, 2001).

Considering that cigarette smoke is a rich source of benzo[a]pyrene and that benzo[a]pyrene is a potent enzyme inducer, it might be inferred that tobacco smoke should induce drug metabolism (Gram, 1997).

2.2.2 Enzyme inhibition

Enzyme inhibition is an extremely common mechanism in the interaction between drugs. Inhibition of the metabolism of drugs subject to biotransformation in the liver often leads to serious adverse effects because of drug accumulation to toxic concentrations (Na-Bangchang and Wernsdorfer, 2001). Inhibition mechanisms include substrate competition, interference with drug transport,

and depletion of hepatic glycogen, enzyme destruction, and functional impairment of enzyme activity by the interacting drugs.

Enzyme inhibition appears to be a dose-related phenomenon. Inhibition of the metabolism of the affected drug begins as soon as sufficient concentrations of the inhibitor appear in the liver. The effect usually reaches the maximum when the new steady-state plasma concentration is achieved. Thus, potentiation of pharmacological effect can occur quickly with drug having a short half-life (Na-Bangchang and Wernsdorfer, 2001).

Enzyme inhibitors are molecules that interact in some way with the enzyme to prevent it from working in the normal manner. There are a variety of types of inhibitors including: nonspecific, irreversible, reversible-competitive and noncompetitive. Certain drug substrates may inhibit cytochrome P450 enzyme activity. Imidazole-containing drugs such as cimetidine and ketoconazole bind tightly to the heme iron of cytochrome P450 and effectively reduce the metabolism of endogenous substrate or other coadministered drugs through competitive inhibition (Katzung, 2001). An inhibitor may or may not be metabolized by the enzyme that it inhibits. Known inhibitors of CYP3A are the macrolide antibiotics erythromycin and troleandromycin, the azole antifungals ketoconazole, itraconazole and fluconazole, the calcium channel entry blockers diltiazem and verapamil and the selective serotonin re-uptake inhibitors fluvoxamine and fluoxetine. It has been reported that grapefruit juice inhibits CYP3A4 in the bowel wall and in the liver. Concomitant ingestion of grapefruit juice with drugs that are a substrate for CYP3A4 reduces their first-pass metabolism, resulting in decreased clearance and increased plasma concentrations of the drugs. Among the drugs reported to be affected by grapefruit juice are the benzodiazepines, the dihydropyridine calcium channel blockers, and the antihistamine terfenadine. All of these compounds are metabolized by cytochrome P450 isoenzyme CYP3A4 (Friedericy and Bovill, 1998).

The three most important CYPs involved in atypical antipsychotic metabolism are CYP3A, CYP2D6 and CYP1A2 (Leon *et al.,* 2005). Metabolism of quetiapine was mainly catalyzed by CYP3A4 and minor role by CYP2D6.

2.3 Bioequivalence study

Approaches to test the bioequivalence (BE) of drug formulations have been evolving over the past two decades (Endrenyi, 1998). Many drugs are marketed by more than one pharmaceutical manufacturer. The study of biopharmaceutics gives substantial evidence that the method of manufacture and the final formulation of the drug can markedly affect the bioavailability of the drug. Because of the plethora of drug products containing the same amount of active drug, physicians, pharmacists and others who prescribe, dispense or purchase drugs must select generic products that produce an equivalent therapeutic effect to the brand product. BE studies provide important information in the overall set of data that ensure the availability of safe and effective medicines to patients and practitioners.

The term BE refers to the comparison of bioavailability of different formulations, drug products or batches of the same drug product (Aulton, 2002). It defines as "the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study." (Chen *et al.*, 2001). Thus two products are bioequivalence if their rate and extents of absorption are the same.

2.3.1 Methods for determining BE

BE may sometimes be demonstrated using an *in vitro* bioequivalence standard, especially when such an *in vitro* test has been correlated with human *in vivo* bioavailability data. In other situations, BE may sometimes be demonstrated through comparative clinical trials or pharmacodynamic studies (Hendrickson, 2006). The requirement could be either an *in-vivo* or an *in-vitro* investigation, as specified by the FDA. The types of BE requirements include the following:

1). An *in-vivo* test in humans.

2). An *in-vivo* test in animals that has been correlated with human *in-vivo* data.

3). An *in-vivo* test in animals that has not been correlated with human

in-vivo data.

4). An *in-vitro* BE standard, i.e., an in-vitro test that has

been correlated with human *in-vivo* bioavailability data.

5). A currently available in-vitro test (usually a dissolution rate test)

that has not been correlated with human *in-vivo* bioavailability data.

2.3.2 BE study design

The study should be designed in such a way that the effects of formulation can be distinguished from other factors. When two formulations are compared, crossover designs are the primary statistical designs for bioavailability and BE studies. Such designs allow for comparison of individual treatments using within subject variation and thus increase the power of the study for a 2×2 crossover design with two treatments and two periods.

A single-dose BE study is generally performed in normal, healthy, adult volunteers. The subject population should be selected carefully, so that product formulations, and not intersubject variations, will be the only significant determinants of BE . A minimum of 12 subjects is recommended, although 18 to 24 subjects are used to increase the data base for statistical analysis. The test and the reference products are usually administered to the subjects in the fasting state (overnight fast for at least 10 h, plus 2 to 4 h after administration of the dose), unless some other approach is more appropriate for valid scientific reasons. These subjects should not take any other medication for one week prior to the study or during the study. The bioavailability is determined by collection of either blood samples or urine samples over a period of time and measurement of the concentration of drug present in the samples. For BE study, both the test and reference drug formulations contain the pharmaceutical equivalent drug in the same dose strength, in similar dosage forms and both are given by the same route of administration (Shargel and Yu, 1999). Generally, a crossover study design is used. Using this method, both the test and the reference products are compared in each subject, so that inter-subject variables, such as age, weight, differences in metabolism, etc., are minimized. Each subject thus acts as his own control. Also, with this design, subjects' daily variations are distributed equally among all dosage forms or drug products being tested.

The subjects are randomly selected for each group and the sequence of drug administration is randomly assigned. The administration of each product is followed by a sufficiently long period of time to ensure complete elimination of the drug (washout period) before the next administration. The washout period should be a minimum of 5 half-lives of the administered drug. A waiting period of one week between administrations is usually an adequate washout period of most drugs.

To avoid bias of the test results, each test subject is randomly assigned one of the two products for the first phase of the study. Once the first assigned product is administered, samples of blood or plasma are drawn from the subjects at predetermined times and analyzed for the active drug moiety or its metabolites as a function of time. The same procedure is then repeated (crossover) with the second product after an appropriate washout period (Aulton, 2002).

Sequential blood samples (about 12 to 18, including a pre-dose sample) shall be drawn at appropriate, specified, and carefully recorded times (to capture increasing and decreasing concentrations during the absorption, distribution and elimination phases). The collections are to continue for about three terminal drug half-lives in order to capture at least 80% of the total area. At least three to four samples need to be obtained from the terminal log-linear phase to derive an acceptable estimate of the terminal constant from linear regression. For long half-life drugs, a truncated AUC (e.g., up to 72 h) is generally considered adequate. Blood samples or the harvested plasma/serum shall be analyzed for the administered drug or metabolites by means of a validated analytical method.

Westlake (1979) summarizes that the selection of sampling times in BE studies with no universal rule is apparent and a pragmatic approach is usually taken. After a single dose of administration, a rule of thumb is that blood samples are drawn at several times during the absorption phase of the drug, then several times near the peak and at relatively fewer times in the elimination phase. Usually, 10-15 total sampling times are employed. For example, for a drug with a half-life of 4-5 h, a typical sampling schedule might be 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 15 and 24 h following administration.

2.3.3 Duration of washout period

The administration of each product is followed by a sufficiently long period of time to ensure complete elimination of the drug (washout period) before the next administration. The washout period should be a minimum of 5 half-lives of the administered drug. A waiting period of one week between administration is usually an adequate washout period of most drugs.

2.3.4 Subjects

The subject population for BE studies should be selected with the aim to minimize variability and permit detection of difference between pharmaceutical products. Therefore, the study should normally be performed with healthy volunteers. The inclusion/exclusion criteria should be clearly stated in the protocol. In general, subjects should be as follow: (Thai FDA, 2006)

- Age between 18-45 y
- Weight within the normal range according to accepted normal values for the body mass index (BMI) 18-25 kg/m^2.
- Should be screened for suitability by means of clinical laboratory tests, an extensive review of medical history, and a comprehensive medical examination
- Before and during of the study, subjects should preferable be non-smokers and without a history of alcohol or drug abuse
- Subjects should not take any other medication prior to the study or during the study

2.3.5 Sample size for BE studies

According to the Committee for Proprietary Medicinal Products (CPMP) guidance, the number of subjects required is determined by the error variance associated with the primary characteristic to be studied (as estimated from a pilot experiment, from previous studies, or from published data), the significance level desired, by the expected deviation from the reference product and by the required power. It should be calculated by appropriate methods and should not be less than 12.

The equation for the approximate sample size calculation for the two one-sided '*t*' test is given below (Liu and Chow, 1992)

$$n \geq [t_{\alpha,2n-2} + t_{\beta,2n-2}]^2 [CV/(\nabla - \theta)]^2$$

where

n	=	number of subjects required per sequence
t	=	the appropriate value from the t distribution
α	=	the significance level
1-β	=	the power
CV	=	co-efficient of variation
∇	=	the BE limit
θ	=	difference between product

2.3.6 Parameters for assessment and comparison of BE

Earlier it has been argued that a BE study is a check on the similarity of the released characteristics of test and reference products. The amount of drug molecules released and speed of the release are therefore the most important parameters. In the *in-vivo* BE study, these characteristics are determined by measuring the following parameters (Figure 5):

2.3.6.1 The maximum concentration (C_{max}): C_{max} is the maximum drug concentration observed in the blood, plasma or serum following a dose of the drug. The C_{max} will usually occur at only a single time point, referred to as T_{max} (Aulton, 2002). It determines the therapeutic efficacy and toxicity of the drug (Na-Bangchang and Wernsdorfer, 2001).

2.3.6.2 The time of peak concentration (T_{max}): The second parameter of importance in assessing the comparative bioavailability of two formulations is the time required to achieve the maximum level of drug in the blood. If changes in the rate of drug absorption will result in changes in the values of both C_{max} and T_{max}. Each product has its own characteristic rate of absorption. When the rate of absorption is decreased, the C_{max} is lower and T_{max} is slower.

2.3.6.3 The area under the plasma concentration-time curve (AUC): AUC is considered representative of the total amount of drug absorbed into the circulation following the administration of a single dose of that drug. Equivalent dose of a drug, when fully absorbed, would produce the same AUC. Thus, two curves are alike in terms of peak height and time of peak.

C_{max} and T_{max} are measures of the rate of systemic availability, whereas the total AUC is a measure of its extent. The AUC is the most important parameter for the assessment of bioavailability or BE of a drug preparation (Na-Bangchang and Wernsdorfer, 2001).
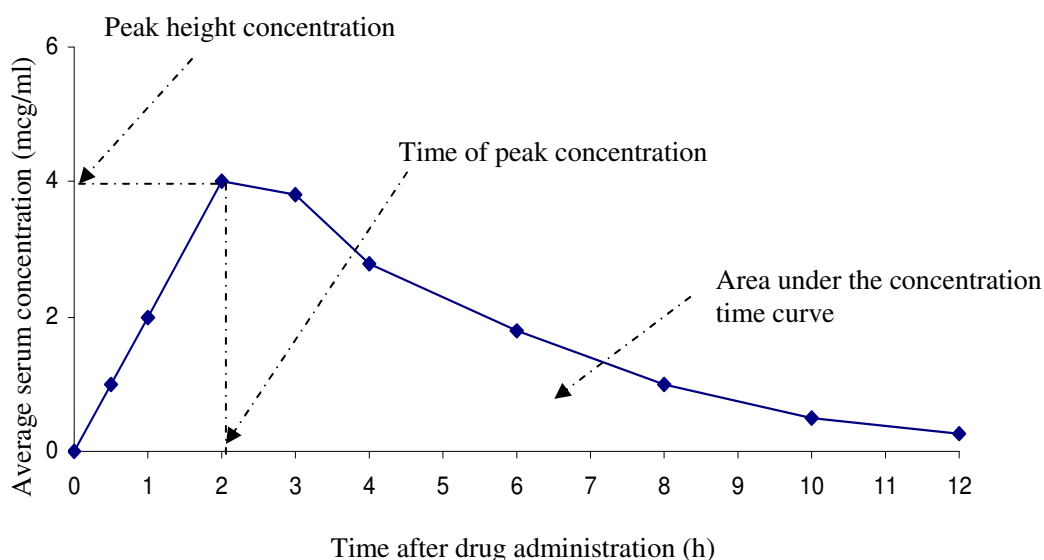

Figure 6. Serum concentration-time curve showing the parameters which are used to determine BE; C_{max}, T_{max}, and AUC (Aulton, 2002).

2.3.7 Evaluation of the data

Analytical method: The analytical method for measurement of the drug must be validated for accuracy, precision, sensitivity, and specificity. Data should be presented in both tabulated and graphical form for evaluation. The plasma drug concentration versus time curve for each drug product and each subject should be available.

Analysis of variance (ANOVA): Data from BE study are commonly evaluated by ANOVA. It is to be used to identify the source contributions by factors including subjects, period, formulation, and potential interactions. The geometric mean ratio together with the ANOVA residual mean error term are used to identify the statistical basis for the 90% confidence interval for the ratio of the population means (test/reference).

The statistical methodology for analyzing these BE studies is called the two one-sided test procedures. Two situations are tested with this statistical methodology. The first of the two one-sided tests determines whether a generic product (test), when substituted for a brand-name product (reference) is significantly may be more bioavailable. The second of the two one-sided tests determines whether a brand-name product when substituted for a generic product is significantly less bioavailable. Based on the opinions of the FDA medical experts, a difference of greater than 20% for each of the above tests is determined to be significant, and therefore, undesirable for all drug products. Numerically, this is expressed as a limit of average test-product /reference-product.

For statistical reasons, all data are log-transformed prior to conducting statistical testing. In practice, these statistical tests are carried out using an ANOVA and calculating a 90% confidence interval (90% CI) for each pharmacokinetic parameter (C_{max} and AUC). The 90% CI for both pharmacokinetic parameters, AUC and C_{max}, must be entirely within the 80% to 125% boundaries cited above. Because the mean of the study data lies in the center of the 90% CI, the mean of the data is usually close to 100% (a test/reference ratio of 1). Different statistical criteria are sometimes used when bioequivalence is demonstrated through comparative clinical trials, pharmacodynamic studies, or comparative in-vitro methodology. Classically, the assessment of BE relies on the concept of average BE. Two drug products, a generic versus the innovator are considered to be bioequivalent if the calculated 90% CI for the ratio of the mean measures of bioavailability (AUC, C_{max}) lies between the predefined BE limits of 0.80-1.25 (Kytariolos *et al.*, 2006). The FDA regulations state that "two formulations whose rate and extent of absorption differ by -20%/+25% or less are generally considered bioequivalent" (Benet, 1999).

There are many studies of antipsychotic BE (Table 3), but no report on BE study of quetiapine in Thailand, this study was therefore to evaluate the BE of quetiapine in Thai healthy volunteers.

Table 3. BE studies of antipsychotics

Drug	Researcher	Year	Place	Dose	Study design	Subjects
Haloperidol	Midha *et al*.	1989	University of Saskatchewan, Canada	5-mg tablet	Three-way crossover	28 healthy male
	Weringh *et al*.	1994	Haarlem Hospital, the Netherlands	100-mg injection	Open, randomized, crossover	15 schizophrenic patients
	Yun *et al*.	2005	Chungnam National University, Korea	5-mg tablet	Single dose, two-way crossover	24 healthy volunteers
	Sight and Sharma	2005	India	5-mg tablet	Two-way, single blind, open-label, two-period, crossover	14 healthy male subjects

Table 3. BE studies of antipsychotic (continued)

Drug	Researcher	Year	Place	Dose	Study design	Subjects
Clozapine	Taesotikul *et al*.	2000	Chiang Mai University, Thailand	100-mg tablet	Single dose, randomized, double blind, two-period crossover	12 healthy volunteers
	Lam *et al*.	2001	University of Texas, USA	100-mg tablet	Randomized, crossover	16 Schizophrenia patients
	Tassaneeyakul *et al*.	2005	Khon Kaen University, Thailand	100-mg tablet	Multiple-dose, randomized, two-way crossover	18 male schizophrenia patients
Risperidone	Gaete *et al*.	2003	Hoapital Clinico de la, Spain	1-mg tablet	Single-dose, randomized, double-blind, two-period	12 healthy volunteers
	Schaick *et al*.	2003	Johnson Pharmaceutical, Belgium	0.5-mg tablet	Open-label, randomized, two-way crossover	37 healthy volunteers
Olanzapine	LI Wen-biao *et al*.	2006	Beijing Anding hospital, China	10-mg tablet	Randomized, two-way crossover	22 male volunteers