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SCIENTIFIC REVIEW

Bioavailability File: Exemestane

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Summary

Exemestane (EXE) is an irreversible aromatase inactivator used for the treatment of postmenopausal women with advanced breast cancer. It is effective in postmenopausal patients with tamoxifen-refractory advanced breast cancer, prolonging time to disease progression and treatment failure and improving survival. The mean plasma elimination half-life of EXE is 24 hours. EXE binds covalently to the active site cytochrome P450, inactivates aromatase and reduces plasma estrogen level. EXE is metabolized in the liver, and cytochrome P450 3A4 (CYP 3A4) is the principal isoenzyme involved in the oxidation of this drug. EXE has been developed for oral administration and is marketed as Aromasin® tablets. In this review, the physicochemical, pharmacological and pharmacokinetic properties and bioavailability of EXE are discussed.

Key Words: *Exemestane, bioavailability, pharmacokinetics, breast cancer, aromatase inhibitor.*

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Biyoyararlanım Dosyası: Ekzemestan

Özet

Ekzemestan (EXE), menopoz sonrası ilerlemiş meme kanserinin tedavisinde kullanılan bir aromataz inaktivatörüdür. Tamoksifen tedavisine cevap vermemiş olan menopoz sonrası hastalarda etkilidir. Hastalığın ilerlemesini geciktirip, tedavinin başarısız olmasını önleyerek, hayatta kalma süresini uzatır. EXE'ın plazmadan eliminasyon yarı ömrü ortalama 24 saattir. EXE sitokrom P450'nin aktif bölgesine kovalent bağlanarak, aromatazı inaktive eder ve plazma östrojen seviyesini düşürür. EXE karaciğerde metabolize olur ve sitokrom P450 3A4 (CYP 3A4), oksidayonunda rol oynayan temel izoenzimdir. EXE oral uygulama için geliştirilmiştir ve Aromasin® tablet olarak pazarlanmaktadır. Bu derlemede, EXE'ın fizikokimyasal, farmakolojik, farmakokinetik özellikleri ve biyoyararlanımı değerendirilmiştir.

Anahtar Kelimeler: *Ekzemestan, biyoyararlanım, farmakokinetik, meme kanseri, aromataz inhibitörü.*

INTRODUCTION

Breast cancer is the leading cause of death among women, with one million new cases in the world each year (1), and one-third of human breast tumors are reported to be hormone-dependent (2,3). It is supported by experimental evidence that estrogens are the most important hormones involved in the growth of these tumors (4,5). Aromatase inhibition is a well-established therapeutic option in postmenopausal, hormone- dependent breast cancer (6). Because of associated side effects in the treatment with first-generation aromatase inhibitors, several new aromatase inhibitors have been developed.

Endocrine agents that are used against breast cancer may be grouped into three main classes: the selective estrogen receptor modifiers, which include drugs like tamoxifen, 'pure' or steroidal antiestrogens (7) and the aromatase inhibitors/inactivators (8-10). Exemestane (EXE) is a third-generation steroidal aromatase inhibitor (11); it has been recently approved by the Food and Drug Administration (FDA) for the treatment of breast cancer and marketed as Aromasin® (12). EXE is a potent inhibitor of peripheral aromatase activity (13,14), and it is the only orally active irreversible steroidal aromatase inactivator (15,16). It is also reported that clinical trials for breast cancer prevention by EXE are available (17).

Following oral administration of radiolabeled EXE, 42% of radioactivity was absorbed from the gastrointestinal

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tract due to its low solubility (18). Preclinical data obtained in rats and dogs, in which EXE was given intravenously (i.v.), indicated that the absolute bioavailability was about 5% (19). EXE plasma levels increased by approximately 40% after a high-fat breakfast (12,18). EXE is extensively metabolized; 10% of the total radioactivity was found after oral administration, which indicates unchanged drug level in plasma, and its metabolites were found to be inactive or show less activity than the parent drug (18). EXE has a mean terminal half-life of 24 h and is eliminated by the liver and kidneys (20).

Treatment with EXE administered orally has been shown to be well tolerated by patients. The most common adverse events of any grade consisted of hot flashes, nausea, fatigue, dizziness, increased sweating, headache, body weight change, vaginal dryness, arthralgias, and myalgias (21-23).

Physicochemical Properties

EXE is a white to slightly yellow crystalline powder with a molecular weight of 296.41. It is a neutral compound with steroidal structure characterized by high lipophilicity. The chemical name of EXE is 6-methylenandrosta-1,4-diene-3,17-dione. Its empirical formula is C20H24O2 and the chemical formula is given in Figure 1 (18,19,23-25).

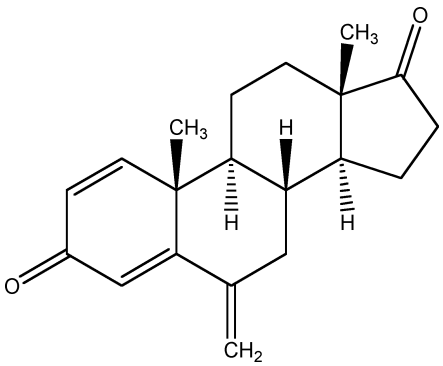

Figure 1 : Chemical structure of EXE.

EXE is freely soluble in N, N-dimethylformamide, soluble in methanol, and practically insoluble in water (18). EXE pH-solubility profile, after stirring for 24 h at 37°C, is shown in Table 1. EXE's melting point is 194°C, it is not hygroscopic, and no polymorphs have been observed (19).

Table 1. EXE pH-solubility profile (19)

Medium	pH	Solubility µg/ml
Water	-	80
Chloride buffer	1,5	86
Acetate buffer	5,5	79
Phosphate buffer	7,4	73

The laboratory synthesis of EXE (Fig. 2) exploited the 6-methylenation of androstenedione with formaldehyde acetal and POCl3. The introduction of the required 1,2-double bond to obtain EXE was then performed by dichlorodicyanobenzoquinone (DDQ) dehydrogenation (24,26).

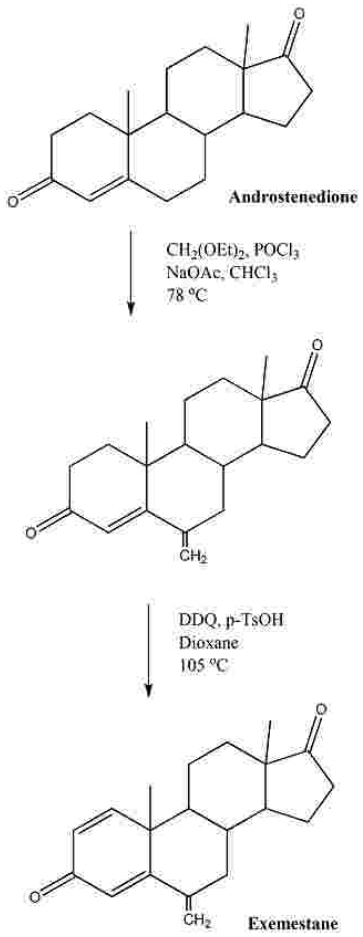

Figure 2 : Laboratory synthesis of EXE (24).

Identification and Quantification Methods

Several high performance liquid chromatography (HPLC) and gas chromatography (GC) methods have been developed with different detecting systems such as UV detection,

mass spectrometric detection, etc. for determination of EXE. Liquid chromatography-mass spectrometry (LC-MS), liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS) and high performance liquid chromatography-radioimmunoassay (HPLC-RIA) methods also have been described for determination of EXE in urine or plasma (27-31).

Reverse phase HPLC (RP-HPLC) is a quantitative method that can be used for in vitro determination of EXE. The chromatographic conditions are given as 247-249 nm at a constant temperature (40°C), with UV detection (32-36). During the HPLC studies, a linear correlation was obtained between absorbance and EXE concentrations over the range of 2.5-50 µg/mL (35).

It is possible to determine EXE in human plasma by RP-HPLC, LC-MS-MS or GC (27,29,36). In LC-MS-MS method, 13C3 EXE was used as an internal standard, and the procedure was found more sensitive compared to the previously published RP-HPLC but less sensitive than the HPLC-RIA method (29). These methods have been optimized to allow the rapid and sensitive detection of EXE in human plasma, but for doping control purposes, it is necessary to test urine. Hence, an LC-MS method was developed for EXE determination in urine. With this method, it is also possible to determine one of EXE's major metabolites, 17-dihydroexemestane (29).

Pharmacology

Mechanism of Action

Breast cancer frequently needs estrogen to proliferate. The major source of estrogen in postmenopausal women is from conversion of androstenedione to estrone by aromatase. Therefore, blocking aromatase represents a potential treatment for breast cancer (37).

Aromatase catalyses the ultimate step in estrogen biosynthesis that converts androgens to estrogens both in pre- and postmenopausal women (38). Biosynthesis of estrogens is represented in Figure 3.

Aromatase is a complex enzyme consisting of two proteins: the aromatase cytochrome P450, hemoprotein, and reduced nicotinamide adenine dinucleotide diphosphate (NADPH) cytochrome P450 reductase, which donates electrons to the P450 aromatase (39,40). EXE is structurally related to the natural aromatase substrate androstenedione and it is initially recognized by the aromatase enzyme as a false substrate and then transformed (through an NADPH-dependent mechanism) to an intermediate, which binds irreversibly to the enzyme, causing its inactivation (41-44). The enzyme inactivating property of EXE has been attributed to the presence of the double bond at C1–C2 (45). Because aromatization is the last step in estrogen biosynthesis, EXE does not disrupt production of other steroids, such as adrenal corticoids (40,43,46).

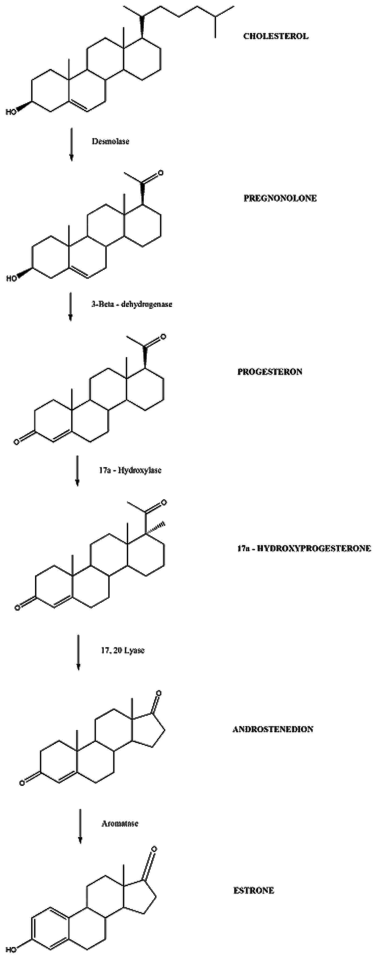

Figure 3 : Biosynthesis of estrogens (26).

Aromatase inactivators such as EXE have been developed primarily for use in postmenopausal women**.** It should be noted that they cannot be used as the sole endocrine treatment since in premenopausal women, the ovaries are the primary site of estrogen production; aromatase inactivators are not capable of blocking ovarian estrogen synthesis completely in premenopausal women (47,48).

Uses and Administration

EXE is indicated for the treatment of postmenopausal women with advanced breast cancer whose disease has progressed after tamoxifen or other antiestrogen therapy (23). It is given orally in tablets after meals to increase plasma level. Dose-ranging studies have demonstrated that dose-related inhibition of estrogen biosynthesis can be achieved with a dose between 2.5 mg (the minimum effective dose) and 25 mg of EXE (41,49,50). Maximal inhibition of peripheral aromatase activity (98%) and estrogen production (85–95%) is observed with an oral dosage of 25 mg (20,51,52).

Even when administered in high doses (100-200 mg/day), EXE is associated with little or no effect on plasma follicle-stimulating hormone, luteinizing hormone, cortisol, or aldosterone levels (11,49,50). No dosage adjustment is necessary in the elderly (53).

Following surgery, postmenopausal patients with hormone-sensitive breast cancer face the risk of disease relapse. Randomized trials have shown that the newer aromatase inactivators such as EXE decrease recurrence and increase the number of women who are suitable for breast conservation when compared to patients treated with tamoxifen (54-56).

Adverse Effects and Precautions

EXE is contraindicated in patients with a known hypersensitivity to the drug or its metabolites. EXE should not be administered to premenopausal women and it should not be coadministered with estrogen-containing agents, as these could interfere with its pharmacologic action (18). The most frequent side effects are hot flashes, nausea, fatigue, dizziness, increased sweating, headache, body weight change, vaginal dryness, arthralgias, and myalgias (21-23). Adverse events occurring during a randomized trial of EXE 25 mg once daily are shown in Figure 4 (57). Management strategies for common complaints associated with EXE are given in Table 2 (54,58-60).

Androgenic events were rarely reported at the recommended therapeutic dosage (42). In a few patients on long-term treatment with 200 mg daily EXE, mild androgenic effects have been reported (61). In randomized phase III trials, no drug-related deaths have been reported due to EXE (57).

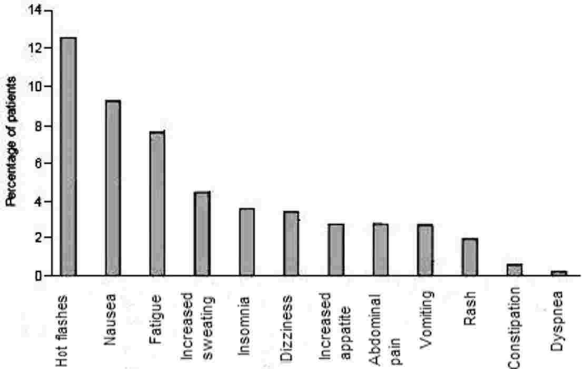

Figure 4 : Adverse events occurring during treatment with 25 mg once daily oral EXE (n = 358) (57).

Table 2. Management strategies for managing adverse events attributed to aromatase inhibitors[54,58-60]

Event	Management Strategy
Bone loss / fractures	➢ Muscle-strengthening exercises ➢ Weight-bearing exercise ➢ Calcium and vitamin D supplementation ➢ Reduced alcohol consumption ➢ Smoking cessation ➢ Use of antiresorptive drugs ➢ DEXA (dual-energy X-ray absorptiometry) scanning to monitor bone health
Arthralgia	➢ Heat ➢ Exercise and physical therapy ➢ Over-the-counter analgesics including NSAIDs (non-steroidal anti-inflammatory drugs) ➢ Antidepressants ➢ Biofeedback methods, visual imagery
Hot flashes	➢ Regular exercise ➢ Relaxation techniques ➢ Use of SSRIs (selective serotonin reuptake inhibitors)
Vaginal dryness	➢ Local estrogen use ➢ Vaginal moisturizers

EXE was generally well tolerated in clinical trials at once daily dosages up to 600 mg, and the maximum tolerated dose was not reached (44,62-65). At a dosage of 25 mg once daily, adverse events considered drug-related were mainly grade 1 to 2 in severity (42,57,62,63). EXE 25 mg once daily was well tolerated in comparison with tamoxifen 20 mg once daily according to data (66). Withdrawal due to adverse effects occurred in 1.7 to 8% of patients (42,57,62,67).

During treatment with EXE 25 mg, no clinically significant effects were reported on blood pressure or heart rate (57,63).

A two-year carcinogenicity study in mice at doses of 50, 150 and 450 mg/kg/day EXE resulted in an increased incidence of hepatocellular adenomas and/or carcinomas in both genders at the high-dose level (18).

Pharmacokinetics and Bioavailability
Absorption

The pharmacokinetics of oral EXE have been studied in both healthy postmenopausal women and postmenopausal women with breast cancer. There are also pre-clinical bioavailability studies in rats and dogs (18,19,21,23,68).

EXE is rapidly absorbed after oral administration and its absorption from the gastrointestinal tract is above 42%. In postmenopausal women with advanced breast cancer, EXE appeared to be more rapidly absorbed than in postmenopausal volunteers (18). It reaches peak plasma concentrations (Cmax) within one to two hours. After maximum plasma concentration is reached, levels decline polyexponentially with a mean terminal half-life of about 24 hours (18,49,69,70).

It was not possible to evaluate the absolute bioavailability in humans due to the absence of a suitable intravenous formulation; however, indirect evidence shows that bioavailability is limited by high first pass effect. The high lipophilicity may be responsible for the high metabolic clearance and extensive first pass effect, which reduces the absolute bioavailability. In preclinical studies with rats and dogs, EXE was given i.v., and the data indicated that the absolute bioavailability was about 5% (19).

In postmenopausal volunteers, mean Cmax after a single oral 25 mg dose of EXE was 17 µg/L, and steady state is reached within seven days of repeated administration (70). EXE shows linear pharmacokinetics over the 1 to 10 mg dose range, although properties were nonlinear at supratherapeutic dosages of 50 to 800 mg (49,71).

The effect of a standard high fat meal on the absorption of EXE administered as a single 25 mg was evaluated, and the results showed that systemic exposure to EXE is increased ($p<0.05$) in the presence of food [area under the plasma concentration time curve (AUC) 41.3 and 29.7 µg/L.h in fed and fasted conditions, respectively]. However, this difference in absorption had no effect on the inhibition of estrone sulphate (75.6 and 69.5% inhibition) (69).

Distribution

EXE is distributed extensively into tissues. EXE is 90% bound to plasma proteins and both albumin and α1-acid glycoprotein contribute to the binding. The fraction bound is independent of the total concentration. The distribution of EXE and its metabolites into blood cells is negligible (18).

Tissue distribution studies in rats indicated tissue to plasma concentration ratios higher than one in all tissues except brain and eyes. Thus, EXE appears to be widely distributed to tissues outside the plasma with the exception of brain tissue (19).

Since the drug could not be given i.v. to humans, the distribution terms were always affected by absolute bioavailability. Findings obtained from preclinical studies, in which the estimates of the volume of the central compartment were calculated after i.v. dosing to rats and dogs, showed volumes of distribution of 4.8 L/kg and 1.8 L/kg, respectively (19).

Metabolization

EXE is extensively metabolized by cytochrome P450 (CYP) 3A4 and aldoketoreductases during the first pass through the gastrointestinal tract and liver following oral administration. The amount of drug excreted unchanged in urine was less than 1% of the dose (19,72).

The metabolites are either inactive or inhibit aromatase activity with lower potency than the parent drug. No metabolites of EXE have shown significant aromatase inhibition except 17-hydro-exemestane, which was 2.6 times less potent than EXE (18,19). In general, the shape of the 17-hydro-exemestane plasma concentration-time profile was similar to that of EXE; however, when measured, concentrations of this metabolite were only about 10% of those of the parent drug. Thus, its contribution to overall pharmacological activity of EXE at therapeutic doses is limited (19).

EXE is metabolized to the 17 β-hydroxy derivative and to other compounds following oxidation of the 6-exomethylene group (38). The initial steps are the reduction of the 17-keto group to give the 17 β-hydroxy steroid, and the oxidation of the methylene group in position 6 with subsequent formation of many secondary metabolites, identified by comparison with the synthetic reference compound (72,73). Metabolites of EXE are shown in Figure 5.

Elimination

After a single oral administration of radio-labelled EXE 100 mg to postmenopausal volunteers, similar amounts of radioactivity (42%) were recovered in urine and feces over a seven-day period (74).

The pharmacokinetics of EXE have been investigated in subjects with moderate or severe hepatic and renal insufficiency. Based on experience with EXE at repeated doses up to 200 mg daily, a moderate increase in non-life-threatening adverse events in patients with both hepatic and renal insufficiency was demonstrated (18,19).

Pharmacokinetic and pharmacodynamic properties of EXE are given in Table 3.

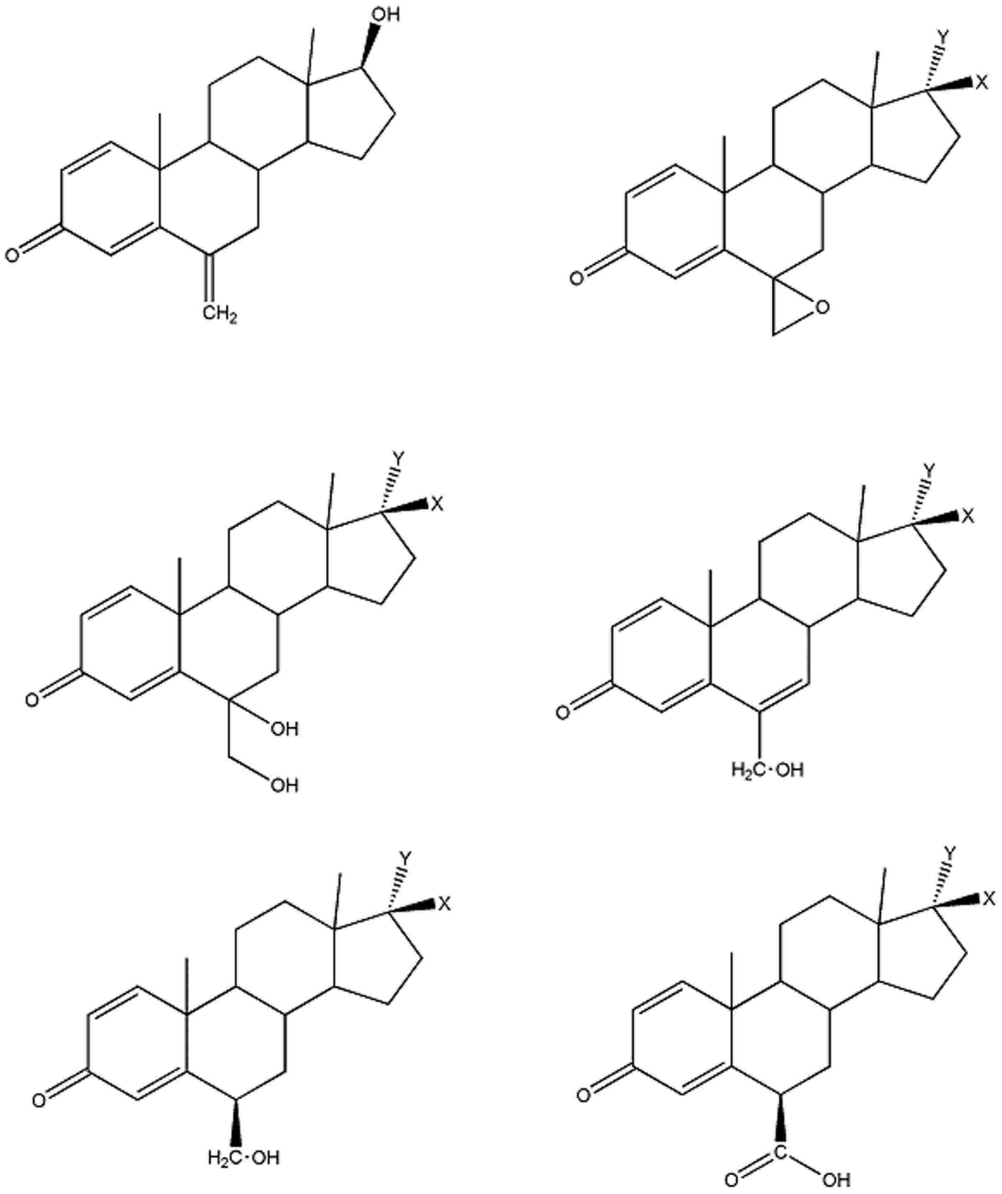

Figure 5 : Metabolites of EXE (X= OH, Y= H; X,Y= O) (26,72,73).

Table 3. Pharmacokinetic and pharmacodynamic properties of EXE (12,18,19,49,69,70)

Property	Value
Bioavailability	5%
Tmax	0.97 h
Absorption	42%
Effect with food	
• T_{max}	⇑ 0.9 h (94%)
• AUC	⇑ 39%
Half-life	24 h
Protein binding	90%
Volume of distribution (V2/F)	19000 L
Metabolism	P450 (CYP) 3A4
Drug interaction risk	Low
Elimination (CL/F)	574 L/h
Pharmacokinetic profile	Linear over the 1 to 10 mg range

Drug-Drug Interactions

EXE does not inhibit any of the major CYP isoenzymes, including CYP 1A2, 2C9, 2D6, 2E1, and 3A4. This would suggest that possible drug-drug interactions due to inhibition of cytochrome P450 by EXE are unlikely.

EXE is metabolized by cytochrome P-450 3A4 (CYP 3A4) and aldoketoreductases. In a clinical pharmacokinetic study, ketoconazole, which is a selective CYP 3A4 inhibitor, showed no significant influence on the pharmacokinetics of EXE. Although there were no other drug-drug interaction studies, significant effects on EXE clearance by CYP isoenzymes inhibitors appear unlikely.

In a pharmacokinetic interaction study of 10 healthy postmenopausal volunteers pretreated with potent CYP 3A4 inducer rifampicin 600 mg daily for 14 days followed by a single dose of EXE 25 mg, the mean plasma Cmax and AUC 0-∞ of EXE were decreased by 41% and 54%, respectively (18,19).

CONCLUSION

Estrogen deprivation is an efficacious approach to the treatment of hormone-dependent breast cancer. EXE is a steroidal irreversible inactivator of aromatase, the enzyme responsible for conversion of steroids such as androstenedione to estrogen. It has been shown to be both safe and effective in the treatment of advanced breast cancer in postmenopausal women who have failed previous hormonal therapy. It also exhibits a long half-life, high potency and selectivity. Available data indicate that EXE does not show cross-resistance with nonsteroidal aromatase inhibitors.

EXE has been developed for oral administration but its bioavailability is 5% due to its low solubility and high lipophilicity. There are ongoing studies to improve its solubility and increase its bioavailability to make this promising agent more effective (35).

It should be noted that metastatic breast cancer is currently an incurable disease. For controlling this life-threatening illness, there are needs for well-tolerated methods. The selectivity, tolerability, and efficacy profiles currently demonstrated with EXE suggest that this agent has a great potential, not only for treatment of postmenopausal breast cancer but also in earlier stages of the disease. Currently, studies are in progress to evaluate EXE as a component of adjuvant and neoadjuvant therapy, as first-line therapy, and as an agent for breast cancer prevention (75).

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