BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **20**: 369–377 (1999)

Pharmacokinetics and Tissue Distribution of Olanzapine in Rats

Manickam Aravagiri*, Yaroslav Teper and Stephen R. Marder
Psychopharmacology Unit, University of California at Los Angeles, VA Greater Los Angeles Healthcare System, Los Angeles, CA, USA

ABSTRACT: The single dose pharmacokinetics of olanzapine in rats, following an oral dose and its distribution in the brain and other tissues after repeated oral and intra-peritoneal (i.p.) administration, were studied. Olanzapine in plasma, brain, liver, lung, kidney, spleen and fat was assayed at predose, 0.25, 0.5, 1, 2, 5, 12, 24, 36, 48 h postoral dose of 6 mg/kg and after daily oral and i.p. doses of 0.25, 1, 3, and 6 mg/kg/day of olanzapine for 15 consecutive days by a sensitive and specific HPLC method with electrochemical detection. Olanzapine was readily absorbed and distributed in plasma and tissues as the peak concentrations were reached within ~45 min after the oral dose. The terminal half-life of olanzapine in plasma was 2.5 h and in tissues it ranged from 3 to 5.2 h. The area under the concentration–time curve (AUC_{last}) was lowest in plasma and largest in liver and lung. The AUC_{last} of olanzapine was eight times larger in brain and three to 32 times larger in other tissues than that in plasma. After repeated oral doses, the plasma and tissue concentrations of olanzapine were generally higher than those after repeated i.p. doses. The liver and spleen had the highest concentrations after oral and i.p doses, respectively. In both cases, the tissue concentrations were four- to 46-fold higher than that in plasma and correlated with administered doses. Likewise, plasma concentrations strongly correlated with the simultaneous brain and tissue concentrations ($r^2 > 0.908$, $p < 0.0001$). On average, the brain levels were 6.3–13.1 and 5.4–17.6 times higher than the corresponding plasma level after oral and i.p. doses, respectively. The tissue to plasma level ratio of olanzapine was higher in other tissues. The data indicated that olanzapine is rapidly absorbed and widely distributed in the tissues of rats after oral and i.p. administration. The plasma concentration appears to predict the simultaneous concentration in brain and other tissues. There was no marked localized accumulation of olanzapine in any of the regions of the rat brain.

Key words: brain–plasma level relationship; HPLC-ECD; olanzapine; pharmacokinetics; rat; regional brain distribution; tissue distribution

Introduction

Olanzapine, a thienobenzodiazepine compound (Figure 1) and a structural congener of clozapine, is one of the newer antipsychotic drugs used in the treatment of schizophrenia and other psychotic disorders. Olanzapine has high affinity to the serotonin 5-HT_{2A} receptor and moderate affinity to dopamine D_1, D_2, D_4, serotonin 5-HT_{2c}, 5-HT_6, 5-HT_7, H_1, α_1-adrenergic and muscarinic receptors [1–6]. Clinical studies indicate that olanzapine is as effective as haloperidol in reducing positive symptoms and superior in reducing negative symptoms [7–9]. The data from a recent PET scan investigation [2,3] showed a near saturation of 5-HT_{2A} receptor binding in schizophrenic patients receiving olanzapine doses as low as 5 mg/day. D_2 occupancy increased with increasing dose of olanzapine. However, it was similar to that observed after risperidone treatment but higher than that seen after clozapine treatment. This type of receptor binding profile with a high 5-HT_{2A} blockade and the avoidance of over blockade of D_2 receptor at common therapeutic doses of an antipsychotic drug are believed to result in a reduced risk of extra pyramidal symptoms [5].

Olanzapine is well absorbed after oral dosing and extensively metabolized to many primary metabolites such as *N*-desmethyl, *N*-oxide, 2-hydroxymethyl, 4′-*N*-glucuronide and 10-*N*-glucuronide metabolites [10,11]. These metabolites are reputed to

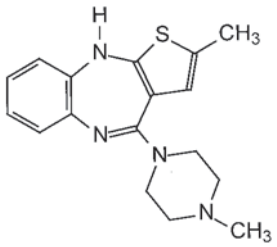

Figure 1. Chemical structure of olanzapine (MW = 312.43)

* Correspondence to: Psychopharmacology Unit, Bldg. 210 # 4, VAGLAHS, Los Angeles, CA 90073, USA. E-mail: kannan@ucla.edu

have no antipsychotic activity. The 10-*N*-glucuronide metabolite of olanzapine is the major circulating metabolite and also the major excreted compound in humans [10]. Thus, olanzapine seems to be the sole active compound responsible for the clinical response. It has been reported that olanzapine concentration in plasma increased with dose [12]. Also, plasma levels correlated well with clinical response [8] and the data revealed that patients with plasma concentrations above 9 ng/mL had a greater likelihood of responding to treatment.

The distribution and the concentration of drugs in brain may be one of the main determinants of a drug's effects. Similarly, with drugs acting on the central nervous system such as antipsychotic drugs, the distribution in brain may play a major role in determining clinical effects. It has been postulated that the brain to plasma level ratios correspond to the potency of antipsychotic drugs, [13–15] indicating that facile distribution leading to higher concentration in brain as compared to plasma concentration may be important for the clinical potency of antipsychotics. However, in the case of newer antipsychotic drugs such as risperidone, this phenomenon was not clearly observed. The brain to plasma concentration ratio after various doses of risperidone was many fold lower than that of an equipotent or even low potency older antipsychotic drugs [16]. Furthermore, with clozapine, a low potency atypical antipsychotic drug, the brain to plasma concentration ratio was high, ~23 after a single i.p. dose [17]. Previous reports have shown that older antipsychotics such as haloperidol and fluphenazine had persistent brain levels and behavioral effects long after (up to 3 weeks) the withdrawal of drug administration [18,19]. These reports suggest that persistence of drugs in brain may be one of the reasons for the persistent behavioral effects. However, with newer antipsychotics such as clozapine there was marked deterioration in mental status within 1 week after clozapine withdrawal [19], probably due to a different distribution pattern of clozapine resulting in sub-therapeutic low concentrations in the brain. In the case of olanzapine, an antipsychotic agent chemically and pharmacologically similar to clozapine, such studies have not been reported. However, it has been shown that olanzapine was readily distributed in all body tissues of the rat including the brain after a single oral dose of 8 mg/kg of ^{14}[C]olanzapine [20] where total radioactivity in the tissues was measured as olanzapine concentration by scintillation counting.

The results from a single oral dose pharmacokinetic study are reported here, in which olanzapine was directly measured in plasma and tissues from 15 min to 48 h postdose and from a study in which olanzapine distribution in the brain and other tissues were measured after the repeated daily oral dose levels to rats. The relationship between plasma and simultaneous brain and tissue olanzapine concentration, the relationship between tissue concentration and administered dose and any localized accumulation in brain regions are discussed.

Materials and Methods

Chemicals

Olanzapine and ethyl olanzapine (internal standard) were generously donated by Lilly Research Labs, Indianapolis, IN, USA. Solvents and chemicals were HPLC grade (Fisher Scientific, Los Angeles, CA, USA) and used without further purification. Deionized water was generated using a ROpure-Nanopure water purification system (Barnstead Co., Boston, MS, USA). All centrifugations were carried out using a refrigerated centrifuge (Centra GP8R, Fisher Scientific, Tustin, CA, USA) at $1725 \times g$.

Animals

The Animal Research Committee and the Research and Development Committee of VA Greater Los Angeles Healthcare System approved experimental procedures using animals. Principles of laboratory animal care (NIH publication no. 85-23, revised 1985) were followed. Male Sprague–Dawley albino rats with body weight ~120 g were purchased from Harlan Sprague–Dawley, San Diego, CA, USA. After 1 week of quarantine from the date of arrival, animals were housed five per cage in a temperature-regulated room (23–25°C) with a 24 h lighting cycle (lights on 06:00–18:00 h). They were gently handled for another week and given a daily dose of water (0.25–0.5 mL) by oral gavage to accustom them to the oral dosing procedure. All animals had free access to food and water at all times.

Experiment and Tissue Collection

The olanzapine doses were made in deionized water acidified with citric acid (~pH 5.5). Rats were given a single oral bolus dose of 6 mg/kg of olanzapine by oral gavage. Four rats for each time-point, predose and 0.25, 0.5, 1, 2, 5, 8, 12, 24, 36, and 48 h postdose, were sacrificed by decapitation. The trunk blood was collected in heparinized glass tubes and whole brain and portions of liver, kidney, lung, fat and spleen were quickly removed and frozen in dry ice.

Rats in the oral treatment group were given daily doses of olanzapine, 0.25 ($n = 5$), 1 ($n = 8$), 3 ($n = 8$) and 6 mg/kg/day ($n = 8$), by oral gavage for 15 consecutive days in the morning (09:00–11:00 h). Similarly, rats in i.p. treatment group were given

3 ($n = 8$) and 6 mg/kg/day ($n = 5$), by i.p. injections for 15 consecutive days in the morning (09:00–11:00 h). Four rats were treated as controls and received water instead for 15 days. On the 15th day, 3 h after the last dose, animals were decapitated and trunk blood from each rat was collected in heparinized glass tubes. Whole brain from four rats in each group receiving 1, 3, and 6 mg/kg/day oral doses and 1 and 3 mg/kg/day i.p. doses were removed and quickly dissected into cerebellum, fronto-parietal cortex, caudate, midbrain (pons-medulla), hypothalamus, hippocampus, olfactory tubercle, and remainder of the brain. These tissue samples were quickly frozen in dry ice. Whole brain from the remaining rats and portions of liver, kidney, lung, fat and spleen from all the rats were quickly removed and frozen in dry ice. Blood samples were centrifuged at 4°C for 10 min at $1725 \times g$. Plasmas were separated and stored at -70°C until analysed. All tissues were homogenized in 5 mL of ice-cold physiological saline (150 mM NaCl) and the homogenates were stored at -70°C until assayed.

Analytical Method

Aliquots of plasma and tissue homogenates were assayed for olanzapine by a modified sensitive high performance liquid chromatography with electrochemical detection (HPLC-ECD) method [12]. Briefly, 100 µL of 100 ng/mL of ethyl olanzapine (10 ng) as internal standard and 0.5 mL of saturated solution of sodium carbonate were added to an aliquot of plasma or tissue homogenates (final pH ~ 10.5, not adjusted). The compounds were extracted with 7 mL of 15% methylene dichloride in pentane solvent mixture. The supernatant solvent layer was removed and evaporated to dryness at 60°C under a slow stream of nitrogen. The residue was dissolved in 150 µL of acetonitrile and analysed by the HPLC-ECD method. The mean absolute recoveries for both compounds were $89 \pm 20\%$ of the total spiked amount. The standard curve was made in saline and consisted of at least six points ranging from 0.25 to 100 ng/mL of olanzapine. Three spiked samples (30–1.5 ng/mL) were made in saline and used as quality control samples to validate the standard curves.

HPLC System

The compounds were separated on an Ultrasphere cyano column (250×4.6 mm i.d., 5 µM particle size, Beckman, San Ramon, CA, USA) and eluted isocratically. The mobile phase consisted of a mixture of an aqueous solution of 45 mM of ammonium acetate (pH ~6.8 not adjusted), methanol and acetonitrile (8:6:84, vol.). The eluted compounds were detected by an electrochemical detector (Decade, using a glassy carbon working electrode. The oxidation potential on the working electrode was $+0.77$ V and the response was measured using a combination of an auxiliary electrode and an Ag/AgCl reference electrode. The detector response was recorded by a chromatographic data collection and data analysis system (Chromquest, Tehrmoquest Corp., San Jose, CA, USA).

Calculations

The concentrations of olanzapine in unknown samples were determined from the standard curve samples analysed on the same day. The standard curve was constructed by plotting the olanzapine to internal standard peak height ratios versus the corresponding concentration of olanzapine in ng/mL of the spiked standard curve samples. Along with each batch of unknown samples, a set of standard curve samples and spiked QC samples were analysed. All the samples were subjected to the same experimental and analytical conditions. Noncompartmental extra vascular model pharmacokinetic parameters were estimated using WinNonlin microcomputer software (Scientific Consulting Inc., Cary, NC, USA). The statistical and graphical analyses were accomplished using commonly available commercial software packages (Microsoft Excel, Microsoft Corp.; Prism, GraphPad Software Inc., San Diego, CA, USA).

Results

The lower limit of determination of olanzapine by the HPLC-ECD method was 0.25 ng/mL when 0.5 mL of the sample was used for analysis (signal to noise > 3). The method is sensitive and precise. The inter- and intra-assay variations were less than 15%. The standard curves were linear over a range of 0.25–100 ng/mL of olanzapine with a correlation coefficient of > 0.997.

Pharmacokinetics

After the single 6 mg/kg oral dose, olanzapine was present in measurable amounts in all tissues up to 36 h postdose except in fat and plasma where it could be measured only up to 12 h, and was present in measurable amounts in liver, lung, and brain up to 48 h postdose, the last time-point. Noncompartmental pharmacokinetic parameters were calculated using WinNonlin software and the results are given in Table 1.

The concentration–time profile of plasma, brain, liver and kidney are given in Figure 2 and they appeared to follow multi-phase elimination pattern. Olanzapine was present in high concentrations in

Table 1. Mean values of noncompartmental pharmacokinetic parameters from plasma and selected tissues of rat following a single oral dose of 6 mg/kg of olanzapine

Tissue	C_{max} (ng/mL or ng/g)	t_{max} (h)	Half-life ($t_{1/2}$) (h)	AUC_{last} (ng · h/mL or ng · h/g)	AUC_{∞} (ng · h/mL or ng · h/g)
Fat	616	0.6	3.0	888	958
Kidney	2821	0.8	4.0	4363	4371
Liver	5146	0.7	5.2	11 310	11 319
Lung	5513	0.6	4.5	11 229	11 247
Plasma	178	0.6	2.5	340	346
Spleen	3592	0.6	3.9	7868	7889
Brain	1162	0.6	5.1	2856	2862

All pharmacokinetic parameters are estimated using a noncompartmental extra vascular input Model (WinNonlin-Model 200).
AUC_{last}, area under the concentration–time curve up to the last assayed postdose time-point.
AUC_{∞}, area under the concentration–time curve extrapolated to concentration at infinite time postdose.
Half-life ($t_{1/2}$), elimination half-life estimated using concentration–time curve from 5 h to last assayed postdose time-point ($n = 4$ at each sampling time).

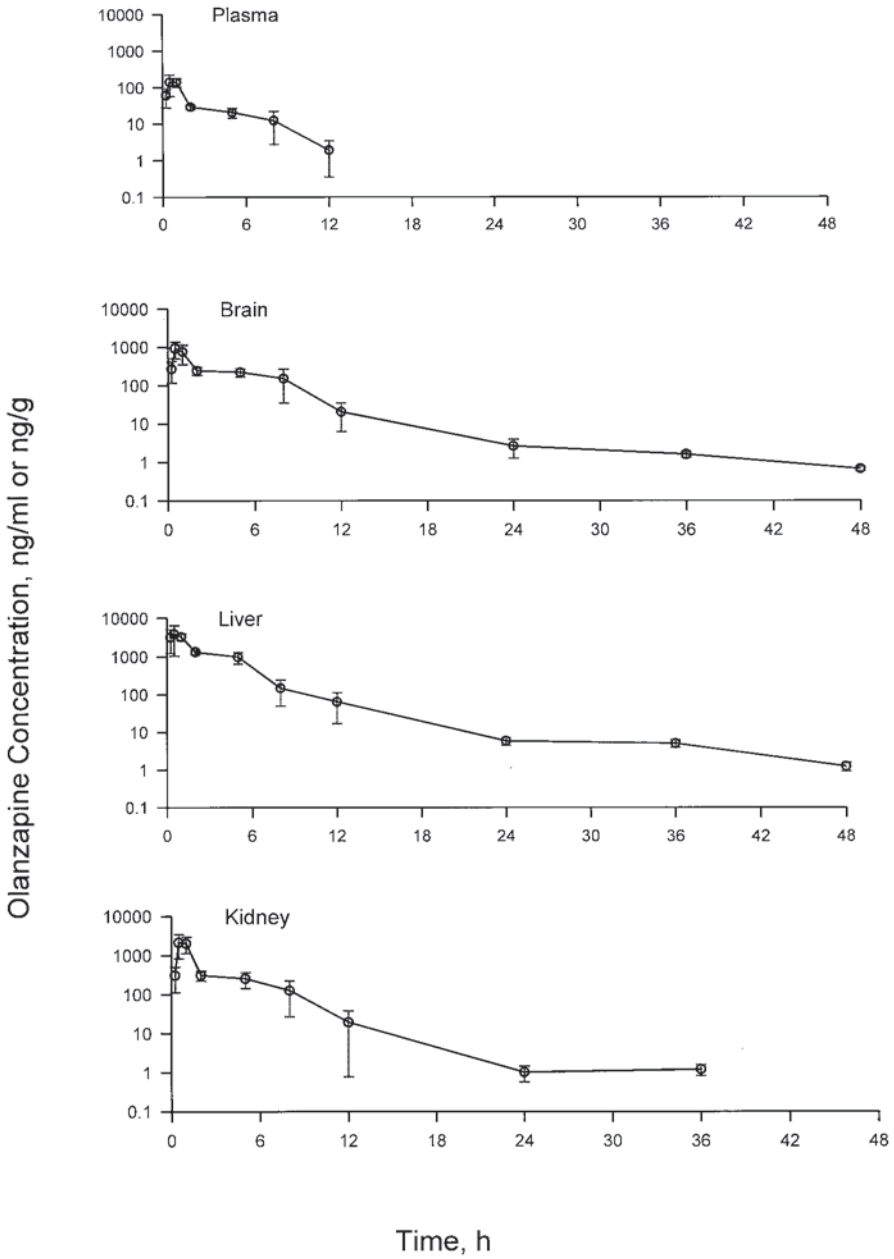

Figure 2. Concentration (mean + S.D.) versus time profiles of olanzapine in plasma, brain, liver and kidney after a single oral dose of

Table 2. The of mean ± S.D. concentrations (ng/g or ng/mL) of olanzapine in brain and other tissues of rat at 3 h after the last daily oral and i.p. doses of 0.25, 1, 3 and 6 mg/kg/day for 15 consecutive days

Tissues	Olanzapine concentration (ng/g or ng/mL) after daily doses of:			
	0.25 mg/kg/day	1 mg/kg/day	3 mg/kg/day	6 mg/kg/day
After oral dose				
Kidney	6.6 ± 3.9	6.7 ± 2.3	40.4 ± 23.7	97.3 ± 45.9
Liver	16.1 ± 5.5	40.3 ± 6.5	351.6 ± 133.0	666.2 ± 339.7
Lung[a]		18.2 ± 4.6	231.9 ± 220.7	454.8 ± 355.2
Plasma	0.7 ± 0.9	0.6 ± 0.3	9.8 ± 3.8	21.9 ± 7.5
Spleen	14.8 ± 4.0	23.9 ± 4.1	225.3 ± 191.7	579.3 ± 184.0
Brain	6.5 ± 6.2	7.3 ± 0.9	51.1 ± 6.9	151.0 ± 87.0
After i.p. dose				
Kidney	5.1 ± 1.2	12.9 ± 4.9	30.2 ± 13.6	84.9 ± 51.1
Liver	7.5 ± 5.1	15.5 ± 5.4	57.2 ± 19.2	143.4 ± 40.8
Lung	ND	26.9 ± 15.6	126.6 ± 31.7	402.1 ± 321.7
Plasma[a]		0.7 ± 0.3	3.2 ± 0.7	12.0 ± 4.9
Spleen	9.7 ± 3.0	42.2 ± 15.4	118.3 ± 58.0	562.1 ± 260.2
Brain	2.6 ± 1.3	9.3 ± 3.8	19.0 ± 9.8	63.1 ± 14.7

ND, nondetectable.
[a] Olanzapine was present in quantifiable amount in only one of the five rats receiving 0.25 mg/kg/day dose ($n = 4$ or 5 rats/dose).

at 15 min and reached peak concentrations (C_{max}) within ~45 min (t_{max}). The terminal half-life ($t_{1/2}$) of olanzapine in plasma was 2.5 h and in other tissues it was slightly longer, varying from 3 to 5.2 h. The area under the concentration–time curve (AUC_{last}) of olanzapine was calculated using the linear trapezoidal rule and it was lowest for plasma and highest for liver and lung followed by spleen, kidney, brain and fat. The AUC_{last} and AUC_{∞} values were similar and the difference was less than 2% except for fat (5%) where olanzapine was not present in quantifiable levels after the 12 h postdose time-point.

Tissue Distribution After Repeated Oral Administration of Various Doses of Olanzapine for 15 Days

Following oral administration, olanzapine was present in measurable amounts in all tissues and the whole brain at all doses except in fat and lung (Table 2). In fat tissue, it was nondetectable at 0.25 and 1 mg/kg/day doses. In lung, olanzapine was present in measurable amount only in one of five rats receiving 0.25 mg/kg/day. At a given dose there were large inter-individual variations observed in the tissue concentrations of olanzapine in all tissues. Olanzapine was present in tissues at higher concentration than in plasma at all dose levels. The liver had the highest concentration followed by the spleen, lung, brain and kidney (Table 2).

Olanzapine levels in brain regions varied widely and the concentrations were very low after the 1 mg/kg/day oral dose, but were present in measurable amounts after 3 and 6 mg/kg/day oral doses (Figure 3). Also, olanzapine was nondetectable in hippocam-

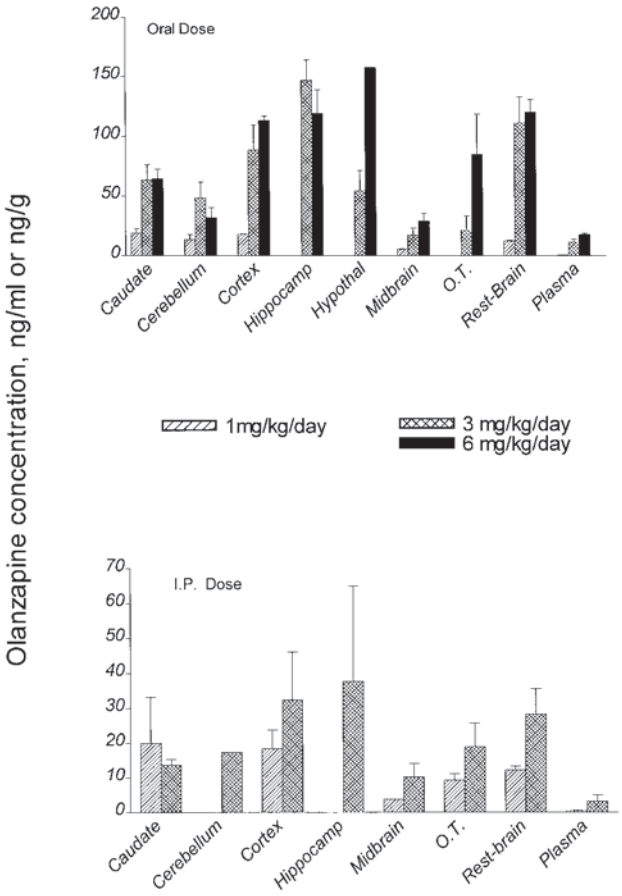

Figure 3. Mean ± S.D. concentration of olanzapine in plasma and in various brain regions of the rat after daily oral doses of 1, 3 and 6 mg/kg/day and daily i.p. doses of 1 and 3 mg/kg/day for 15 consecutive days. The olanzapine concentrations were determined at 3 h after the last dose ($n = 4$ rats/dose). OT, olfactory

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.