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Ketoconazole-Associated Liver Injury in Drug-Drug Interaction Studies in Healthy Volunteers

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Abstract

Ketoconazole is a potent CYP3A inhibitor in vivo, and frequently serves as an index CYP3A inhibitor in drug-drug interaction (DDI) studies with healthy volunteers. Limitations restricting the use of systemic ketoconazole in such studies have been recently imposed by regulatory agencies in the United States, the European Union, and elsewhere. A risk of ketoconazole-associated liver injury in the context of DDI studies was cited as the primary justification for these measures. To evaluate the basis for these restrictions, we analyzed a series of published DDI studies identified from a review of existing literature. The study set consisted of 53 DDI studies, and included 971 healthy volunteers with systemic ketoconazole exposure in addition to the victim drug under study. Ketoconazole-associated abnormalities in serum chemistry values indicative of liver injury were observed in 4 subjects, representing a prevalence of 0.41% within the study population. There were no major adverse reactions or instances of hepatic failure. All abnormalities indicative of liver injury resolved upon discontinuation of ketoconazole treatment. The findings from this review do not support restriction of ketoconazole as an index CYP3A inhibitor in DDI studies involving healthy volunteers.

Keywords

ketoconazole, liver injury, healthy volunteers, drug-drug interaction studies, CYP3A

The azole antifungal agent ketoconazole is a potent inhibitor of human CYP3A isoforms, and is commonly used as an index CYP3A inhibitor in drug-drug interaction (DDI) studies involving healthy volunteers.[1–3] On July 26, 2013, the United States Food and Drug Administration (FDA) and the European Medical Agency (EMA) released directives imposing limitations on the use of oral ketoconazole as a first-line antifungal treatment, citing a potential risk for acute hepatic injury as the primary concern, along with a risk of adrenal insufficiency and drug interactions.[4,5] These restrictions were limited to systemic ketoconazole use, and did not apply to topical formulations.[4] The communication had clear ramifications for clinicians, but the implications for investigators conducting DDI studies were not immediately evident. On October 16, 2013, the FDA further recommended against exposure of study subjects in DDI studies to ketoconazole, stating that "drug companies and researchers [should] avoid using oral ketoconazole in drug interaction studies."[6] Regulatory agencies in Australia and China followed, discontinuing the oral formulation of ketoconazole in late 2013 and 2015, respectively.[7,8]

No specific data analysis was provided to support the regulatory decision to single out ketoconazole as having hazards beyond what is associated with any azole antifungal.[2,9] Presumably, data from the FDA Adverse Events Reporting System (FAERS) was interpreted by regulatory agencies as supporting the restrictions imposed on the clinical use of ketoconazole as an antifungal agent. However, a recent FAERS analysis by Raschi et al. indicated that essentially all azole antifungal agents, including ketoconazole, are associated with a risk of liver injury.[9]

Standard clinical ketoconazole regimens generally consist of 200 mg per day in adults, and treatment durations may exceed 6 months. In a recent review of ketoconazole-associated hepatotoxicity, liver injury appeared more likely in treatment regimens that exceeded 30 days.[10] However, DDI studies in healthy volunteers typically use systemic ketoconazole schedules of 200 or 400 mg per day for 5 to 14 days. The FDA prohibition assumes that the use of oral ketoconazole for the duration of such a study carries a risk of liver injury similar

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to that associated with clinical use of longer duration. The present paper reviews recently published DDI studies to evaluate the prevalence of ketoconazole-associated abnormalities indicative of liver injury.

Methods

Inclusion and Exclusion Criteria

A review of the literature was conducted through PubMed using search terms "ketoconazole," "CYP3A," and "inhibitor." Studies selected for review were clinical DDI trials that met the following criteria: (1) Oral ketoconazole was given as the sole perpetrator drug, along with a victim drug. Exceptions were made for studies that utilized multiple perpetrator drugs if separate safety analyses were performed for each drug. (2) Subjects were healthy volunteers with no prior significant medical conditions as defined by the investigators of each trial, including any specific factors that might predispose to liver injury. Studies that included subjects with liver disease, a history of alcohol abuse, or other predisposing factors were not used in the analysis. (3) Commonly used serum chemistry indices of liver injury, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin, were measured at baseline and after ketoconazole exposure, and the relevant results were reported. (4) Studies were published between 2005 and 2015.

The PubMed search strategy was assumed to yield a random selection of existing DDI studies. Analysis of the literature was limited to published studies and did not include unpublished reports to which we did not have access.

Analysis of Data

For each study, the ketoconazole dosing schedule, the daily oral dose, the duration of exposure, and the total exposure were recorded. Any clinically relevant changes in serum chemistry or other adverse events associated with ketoconazole were documented. Evidence of liver injury was noted if the subject experienced notable adverse symptoms, or if any indicative serum parameter was greater than the upper limit of normal (ULN), or was ≥30% above the baseline value, in accordance with FDA guidelines for oral ketoconazole dosing as stated in the product label.[11]

Results

Characteristics of the Studies Reviewed

The selected study set consisted of 53 separate DDI studies taken from 46 publications between 2005 and 2015, which included 971 subjects with systemic ketoconazole exposure[12–57] (Table 1). A majority of the studies were conducted at study centers in the United States and Germany (Table 2). There was no apparent association between study location and occurrence of ketoconazole-associated hepatic injury. The median total systemic ketoconazole exposure was 2800 mg, and the median daily exposure was 400 mg. The study period duration varied, ranging from a single day of ketoconazole exposure to 28 days. The median duration of exposure was 7 days.

Occurrence of Ketoconazole-Associated Abnormalities

Of the 971 subjects included in the review, 6 subjects in 6 separate DDI studies had clinically relevant changes in laboratory parameters during or after the DDI study, possibly indicative of liver injury. Affected individuals are referred to as subjects A-F (Table 3). Abnormal clinical chemistry values in 3 subjects (A, E, and F) were considered likely to be ketoconazole treatment-emergent and possibly indicative of acute liver injury. Mildly elevated ALT and AST in another subject (D) may or may not have been attributable to ketoconazole. Elevated serum ALT levels in the remaining 2 subjects were considered related to the victim drug as opposed to ketoconazole. The 4 cases in which ketoconazole was probably or possibly implicated represent 0.41% of all subjects included in the study set. None of the studies in which abnormal serum chemistry was observed were outliers in terms of daily ketoconazole dose, study duration, or total ketoconazole exposure, and there was no evident association between any of the aforementioned variables and serum chemistry abnormalities. There was no reported instance of irreversible hepatic dysfunction or liver failure. All abnormalities in clinical chemistry values were reversible, and resolved on completion of ketoconazole treatment. No serious sequelae or deaths were reported.

Consideration of Individual Cases

Subject A was withdrawn on day 14 of a study by Lahu et al[19] which evaluated the possible DDI between ketoconazole and roflumilast, a phosphodiesterase-4 inhibitor. The subject had received 200 mg oral ketoconazole daily for the 6 previous consecutive days. ALT levels reached 190 U/L (ULN = 60 U/L) on study day 14, at which point the subject was withdrawn from treatment. ALT levels peaked at 273 U/L (>4.5 times ULN) on study day 21, and returned to the normal range by day 35. Following withdrawal from the study, the subject's AST levels were elevated to 1.5 to 2 times ULN; however, exact AST values were not reported. AST levels returned to the normal range by study day 28. The findings were considered to be consistent with transient liver injury associated with ketoconazole.

Kotsuma et al[21] reported 2 treatment-emergent adverse effects and an increase in serum ALT levels in

Table 1. DDI Studies Reviewed in the Present Analysis

Reference Number	Victim Drug	Publication Year	N	Ketoconazole Daily Dose (Mean, mg)	Duration of Exposure (Days)	Total Exposure (mg)
12	Solifenacin	2005	16	200	20	4000
13	Dexloxiglumide	2005	23	200	6	1200
14	Prasugrel	2006	18	400	9	3600
14	Clopidogrel	2006	18	400	9	3600
15	Cinacalcet	2007	24	400	7	2800
16	Cinitapride	2007	16	400	7	2800
17	Praziquantel	2007	10	400	5	2000
18	Aliskiren	2008	20	400	4	1600
19	Roflumilast (1)	2008	26	200	1	200
19	Roflumilast (2)	2008	16	400	13	5200
20	Temsirolimus	2008	17	400	7	2800
21	Pactimibe	2008	18	400	7	2800
22	Maraviroc	2008	12	400	9	3600
23	Maribavir	2008	20	400	1	400
24	Ciclesonide	2008	14	400	7	2800
25	Ambrisentan	2009	16	400	7	2800
26	Saquinavir/Ritonavir (1)	2009	29	200	14	2800
26	Saquinavir/Ritonavir (2)	2009	13	200	20	4000
27	Udenafil	2010	12	400	3	1200
28	Sotrastaurin	2010	18	400	6	2400
29	Tolvaptan	2011	17	200	3	600
30	Tamsulosin	2011	23	400	5	2000
31	Alitretinoin	2011	16	200	3	600
32	Neratinib	2011	23	400	5	2000
33	Bosutinib (1)	2011	24	400	5	2000
34	Nilotinib	2011	25	400	6	2400
35	BMS 690514	2012	17	400	9	3600
36	Vilanterol Tri	2012	20	400	6	2400
36	Fluticasone Furoate + VI	2012	18	400	11	4400
37	Safinamide	2012	14	400	6	2400
38	Bosutinib (2)	2012	48	400	5	2000
39	Buprenorphine	2012	15	382	11	4200
40	Ruxolitinib	2012	16	400	4	1600
41	Risperidone	2012	10	200	3	600
42	Midostaurin	2013	27	400	10	4000
43	Macitentan	2013	10	400	24	9600
44	Rivaroxaban (1)	2013	12	200	4	800
44	Rivaroxaban (2)	2013	20	400	5	2000
45	Ponatinib	2013	22	400	5	2000
46	Vorapaxar	2013	12	400	28	11,200
47	Lenvatinib	2014	18	400	17	6800
48	GSK239512 (1)	2014	6	400	9	3600
48	GSK239512 (2)	2014	16	400	9	3600
49	Dabrafenib	2014	16	400	4	1600
50	Apixaban	2014	18	400	6	2400
51	Vilazodone (1)	2014	15	200	14	2800
51	Vilazodone (2)	2014	21	200	14	2800
52	PF-04449913	2014	13	400	7	2800
53	CG100649	2014	26	400	5	2000
54	Apremilast	2014	18	400	7	2800
55	Teneligliptin	2014	16	400	5	2000
56	Fentanyl	2015	16	400	2	800
57	Cabozantinib	2015	27	400	28	11,200

subject B, although exact ALT levels were not provided. This study evaluated the possible DDI of ketoconazole with pactimibe, an ACAT-1 inhibitor that failed to reach the market. The ketoconazole dosing schedule was 400 mg daily for 1 week. The reported abnormalities were not exclusive to the ketoconazole treatment period, and resolved without any action taken. These events were considered to be unrelated to ketoconazole.

Table 2. Origin of the Reviewed DDI Studies

Country of Origin	Number of Studies	Number of Subjects	Ketoconazole Exposure Duration (Mean, Days)	Total Ketoconazole Exposure (Mean, mg)	Instances of Ketoconazole-Associated Serum Chemistry Abnormalities
United States	24	455	9	3322	1
Germany	12	218	6	2133	1
Netherlands	4	100	11	3300	0
France	2	42	17	3400	0
South Korea	2	38	4	1600	0
United Kingdom	2	28	8	3200	0
Australia	2	22	9	3600	2
Canada	1	22	5	2000	0
Thailand	2	20	4	1300	0
Spain	1	16	7	2800	0
Israel	1	10	24	9600	0

Table 3. Summary of Biochemical Abnormalities Observed in the Reviewed Studies

Study Reference Number	Affected Subject	Event	Assessment of Causality	Outcome
19	A	Elevated serum ALT (273 U/L, ULN = 60 U/L) and AST (exact values not reported, 1.5-2 × ULN)	Ketoconazole-associated	ALT and AST levels returned to normal range 21 days after treatment discontinuation
21	B	Elevated serum ALT (exact values not reported)	Unrelated to ketoconazole	ALT levels spontaneously returned to normal range
28	C	Elevated serum ALT (74 U/L, ULN = 60 U/L)	Unrelated to ketoconazole	ALT levels returned to normal range within 60 days after treatment discontinuation
47	D	Elevated serum ALT and AST (exact values not reported, mild severity)	Possibly ketoconazole-associated	ALT levels spontaneously returned to normal range
48	E	Elevated serum ALT (144 U/L, ULN= 60 U/L)	Ketoconazole-associated	ALT levels returned to normal range after treatment discontinuation
48	F	Elevated serum bilirubin (40 mmol/L, ULN=17 mmol/L)	Ketoconazole-associated	Bilirubin levels returned to normal range after treatment discontinuation

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal.

In a study investigating the kinetics of sotrastaurin with CYP3A inhibition conducted by Kovarik et al,[28] elevated ALT levels were observed in subject C after completion of the ketoconazole dosing regimen. Treatment consisted of 200 mg oral ketoconazole twice daily for 6 days, along with a single dose of sotrastaurin on study day 4. ALT levels were reported at 74 U/L (>20% elevation over ULN) at the end of the study period. The investigators were not able to reassess the affected subject for 60 days, at which point ALT levels were within the reference range. The investigators considered these abnormalities likely related to sotrastaurin.

Elevated serum ALT and AST levels were reported in subject D in the study by Shumaker et al.[47] The trial evaluated the kinetics of lenvatinib, a novel VEGFR2 and VEGFR3 kinase inhibitor, when given with 400 mg ketoconazole for 17 consecutive days. Exact ALT and AST serum levels were not presented, but the investigators considered the changes to be of mild severity. The relation to ketoconazole treatment was not established.

In a study conducted by Xu et al,[48] GSK239512, an experimental H_3-receptor antagonist, was the victim drug. Peak ALT levels of 144 U/L (>2 times ULN) were reported in subject E. ALT levels began to decrease at the termination of ketoconazole treatment, and returned to normal. In a separate cohort of the same study, subject F experienced transient asymptomatic hyperbilirubinemia (40 mMol/L, ULN = 17 mMol/L). Both study cohorts received 400 mg oral ketoconazole daily for 9 days. The investigators considered the abnormalities in both cases to be related to ketoconazole.

Discussion

Less than half of 1% (0.41%) of subjects included in the present review had evidence of ketoconazole-associated hepatic injury. In all cases the findings consisted of asymptomatic abnormalities in commonly used serum chemistry indices of liver injury, and all resolved on treatment discontinuation with no specific medical intervention. This may reflect the relatively short

treatment duration and low overall ketoconazole exposure in DDI studies as compared to a clinical treatment course. The incidence of ketoconazole-associated liver injury found in this review is consistent with a recent report by Lo Re et al.[58]

Ketoconazole produces rapid and reversible CYP3A inhibition, and DDI studies with ketoconazole preexposure durations as short as 1-2 days produce maximal inhibition of CYP3A metabolic activity.[2,3,59,60] In the present analysis, subjects who experienced probable or possible ketoconazole-associated abnormalities (A, D, E, and F) had been exposed to ketoconazole for longer than the median study length of 1 week (13, 17, 9, and 9 days, respectively) prior to discontinuation of ketoconazole treatment. All studies involved healthy subjects without hepatic disease or other predisposing factors, and excluded individuals with a history of alcohol abuse.

Our review has the limitation that we assumed the studies we identified to be representative of the larger data base. In addition, we did not have access to unpublished data. Nonetheless, we evaluated reports of close to 1000 healthy volunteers who received ketoconazole in the course of clinical DDI studies. The findings indicate that systemic ketoconazole use in this context carries minimal risk of hepatic injury.

A number of alternative index CYP3A inhibitors have been proposed, for use in DDI studies. Itraconazole and clarithromycin have been proposed, but do not produce in vivo CYP3A inhibition comparable to ketoconazole.[2,61–63] Itraconazole is associated with incidence rates of hepatic injury similar to ketoconazole.[2,9,58] Ritonavir produces in vivo CYP3A inhibition similar to or greater than ketoconazole, and is the most appropriate perpetrator drug to serve as an alternative to ketoconazole in DDI studies of healthy volunteers.[2,63,64] Cobicistat is closely related to ritonavir,[65] and is another option to serve as an index CYP3A inhibitor.[61,63,64]

Conclusions

Ketoconazole should continue to serve as the standard CYP3A inhibitor in DDI studies involving healthy volunteers. The risk of liver injury in this context is minimal, and can be further mitigated by using short durations of ketoconazole exposure.

Disclosures

The authors have no disclosures to report.

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