Does the circulating ketoconazole metabolite N-deacetyl ketoconazole contribute to the drug-drug interaction potential of the parent compound?

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ABSTRACT

Ketoconazole is a strong inhibitor of cytochrome P450 3A4 (CYP3A4) and of P-glycoprotein (P-gp) and is often used as an index inhibitor especially for CYP3A4-mediated drug metabolism. A preliminary physiologically based pharmacokinetic (PBPK) model for drug-drug interactions indicated possible involvement of a metabolite to the perpetrator potential of ketoconazole. Still unknown for humans, in rodents, N-deacetyl ketoconazole (DAK) has been identified as the major ketoconazole metabolite. We therefore investigated in vitro, whether DAK also inhibits the human CYPs and drug transporters targeted by ketoconazole and quantified DAK in human plasma from healthy volunteers after receiving a single oral dose of 400 mg ketoconazole. Our data demonstrated that DAK also inhibits CYP3A4 (2.4-fold less potent than ketoconazole), CYP2D6 (13-fold more potent than ketoconazole), CYP2C19 (equally potent), P-gp (3.4-fold less potent than ketoconazole), breast cancer resistance protein (more potent than ketoconazole) and organic anion transporting polypeptide 1B1 and 1B3 (7.8-fold and 2.6-fold less potent than ketoconazole). After a single oral dose of 400 mg ketoconazole, maximum concentrations of DAK in human plasma were only 3.1 % of the parent compound. However, assuming that DAK also highly accumulates in the human liver as demonstrated for rodents, inhibition of the proteins investigated could also be conceivable in vivo. In conclusion, DAK inhibits several CYPs and drug transporters, which might contribute to the perpetrator potential of ketoconazole.

1. Introduction

Ketoconazole represents a very effective antimycotic drug, which acts like other imidazole derivatives by inhibiting the synthesis of the main membranous steroid ergosterol in fungi (Hume and Kerkering, 1983). In humans, it inhibits the synthesis of other sterols such as cortisol and is therefore also administered to treat endogenous Cushing’s syndrome (Pivonello et al., 2015). In addition, it was mandatory used as an index inhibitor of CYP3A4-mediated drug metabolism due to its strong inhibitory potential for the drug-metabolising enzyme cytochrome P450 3A4 (CYP3A4) (Outeiro et al., 2016).

Although already licensed about 40 years ago, the metabolism of ketoconazole in humans is still not fully elucidated. Early publications state extensive hepatic microsomal metabolism (Hume and Kerkering, 1983; Heel et al., 1982; Daneshmed & Warnock, 1988) and later studies found numerous metabolites in vitro after incubation of human liver microsomes with ketoconazole (Fitch et al., 2009; Kim et al., 2017), but surprisingly there are no in vivo data demonstrating, which metabolites are formed in humans and which enzymes are involved. In contrast, studies in mice and rats clearly show several metabolites formed in vivo, of which N-deacetyl ketoconazole (DAK) has been identified as the major one (Whitehouse et al., 1990, 1994a; Rodriguez and Acosta, 1997a,b).

A preliminary physiologically based pharmacokinetic (PBPK) model for drug-drug interactions suggested that the inhibitor action cannot be fully explained by the parent compound (Lehr et al., unpublished data) and is possibly related to metabolites with inhibitory potential, as seen for itraconazole (Hanke et al., 2018).

Thus, assuming that DAK also represents a major metabolite in humans, it is conceivable that this metabolite might contribute to the...
drug-drug interaction potential of ketoconazole. We, therefore, investigated whether DAK inhibits the same CYPs and drug transporters that are also targeted by ketoconazole: CYP3A4, CYP2C19, CYP2D6, P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and organic anion transporting polypeptides (OATP) 1B1 and 1B3. In addition, we quantified DAK in human plasma from healthy volunteers after receiving a single oral dose of 400 mg ketoconazole to evaluate whether therapeutic concentrations might be sufficient for inhibition of CYPs and drug transporters.

2. Materials and methods

2.1. Materials

Cell culture media and supplements, phosphate buffered saline (PBS), foetal calf serum (FCS), rifampicin, vincristine, doxorubicin, quinidine, omeprazole, and the Cytotoxicity Detection Kit (LDH) were purchased from Sigma-Aldrich (Taufkirchen, Germany). DAK, quercetin, quercetin-3-glucuronide, and the Cytotoxicity Detection Kit (LDH) were purchased from Promega (Madison, WI, USA). DAK and ketoconazole-D₄ were purchased from Biozol (Eching, Germany). Acetonitrile and formic acid were purchased from Biosolve (Valkenswaard, The Netherlands), tert-butyl methyl ether and fumitremorgin C (FTC) from Merck (Darmstadt, Germany).

2.2. Inhibition of CYP2C19, CYP2D6, and CYP3A4

Direct inhibition of CYPs by ketoconazole and its metabolite was quantified with the P450-Glo™ CYP2C19 Screening System, the P450-Glo™ CYP2D6 Screening System, and the P450-Glo™ CYP3A4 Assay according to the corresponding manufacturer’s instructions. The principle of these assays is based on the conversion of a luminogenic substrate (luciferin-H EGE for CYP2C19 (10 μM), luciferin-ME EGE (30 μM) for CYP2D6, and luciferin-IPA (3 μM) for CYP3A4) to luciferin by the respective enzyme. After starting the reaction by adding the NADPH regeneration system, plates were incubated at 37 °C for 12 min (CYP2C19), 30 min (CYP2D6), and 10 min (CYP3A4), respectively. After adding the luciferin detection reagent and further incubation for 20 min at room temperature, luminescence was measured by a GloMax luminometer (Promega Corporation, Madison, WI, USA). For ketoconazole, eight concentrations (0.001–10 μM) in duplicates were investigated. For DAK, eight concentrations (0.0125–25 μM) in technical triplicates were investigated. All experiments were conducted in biological quadruplicates or quintuplicates. Omeprazole and quinidine were used as control inhibitors for CYP2C19 and CYP2D6, respectively.

2.3. Cytotoxicity assays

Toxic effects on cells can negatively influence transporter inhibition assays. Thus, ketoconazole and DAK were tested for their cell-toxic effects using the Cytotoxicity Detection Kit (LDH) according to the manufacturer’s instruction. While ketoconazole was not toxic in all cell lines up to 100 μM, DAK was toxic at higher concentrations, and the toxic concentrations differed between the cell lines used. In all inhibition assays, DAK was used only at concentrations not exceeding 30% cytotoxicity.

2.4. P-gp inhibition assay in the LLC cell system (calcein efflux assay)

To investigate P-gp inhibition, calcein assays were performed as published previously (Weiss et al., 2003) using the t-MDR1 cell line over-expressing human P-gp and the corresponding parental cell line LLC-PK1 (Schinkel et al., 1996) kindly supplied by Dr. A. H. Schinkel (The Netherlands Cancer Institute, Amsterdam, The Netherlands). LLC-PK1 and t-MDR1 cells were cultured under standard cell culture conditions with medium M199 supplemented with 10% FCS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin sulphate. To maintain P-gp/ABC1 expression, the culture medium for t-MDR1 was supplemented with 0.64 μM vincristine. On the day before using the cells for the calcein assay, both cell lines were fed with vincristine-free culture medium.

Ketoconazole was tested from 0.05 to 100 μM. Due to toxicity, DAK could only be tested from 0.01 up to 50 μM. Quinidine was used as a control inhibitor. Each concentration was tested in technical octuplets and each experiment was performed in biological quadruplicates or quintuplicates. Possible quenching effects of the compounds could be excluded, because no effects on calcein fluorescence were observed for non-toxic concentrations in the parental cell line LLC-PK1.

2.5. P-gp inhibition assay in the P388 cell system (calcein efflux assay)

Because ketoconazole not only increased intracellular calcein fluorescence in the P-gp over-expressing cell line t-MDR1, but also in the parental cell line LLC-PK1, concurrent inhibition of MRP was assumed (Fröhlich et al., 2004; Lindenmaier et al., 2005); we therefore also performed the calcein assay in a second cell system without MRP expression: The monocytic murine cell line P388 and the P-gp over-expressing cell line P388/dx (Boesch et al., 1991), kindly provided to us by Dr. D. Ballinari (Pharmacia & Upjohn, Milano, Italy). P388 cells were cultured under standard cell culture conditions with RPMI 1640 medium supplemented with 10% FCS, 2 mM glutamine, 500 mM β-mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin sulphate. For maintaining P-gp expression, the culture medium for P388/dx contained 0.43 μM doxorubicin. One day before the inhibition assay, both cell lines were placed on doxorubicin-free culture medium.

The assay was performed as described previously (Fröhlich et al., 2004). Ketoconazole was tested from 0.05 to 100 μM. Due to toxicity, DAK could only be tested from 0.01 up to 50 μM. Quinidine was used as a control inhibitor. Each concentration was tested in technical octuplets and each experiment was performed in biological quadruplicates. Possible quenching effects of the compounds could be excluded, because no effects on calcein fluorescence were observed for non-toxic concentrations in the parental cell line P388.

2.6. BCRP inhibition assay (flow cytometric pheophorbide A efflux assay)

BCRP inhibition was tested in MDCKII-BCRP over-expressing human BCRP (Pavek et al., 2005) and compared to the parental cell line MDCKII using the fluorescent substrate pheophorbide A and flow cytometry as described previously (Weiss et al., 2007). Cells were kindly provided by Dr. A. H. Schinkel (The Netherlands Cancer Institute, Amsterdam, The Netherlands) and cultured under standard cell culture conditions in DMEM containing 10% FCS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin sulphate.

Ketoconazole was tested from 0.5 to 100 μM and DAK from 0.5 to 75 μM (due to toxicity at higher concentrations). FTC was used as a control inhibitor. Each experiment was conducted in biological triplicates or quadruplicates as described in detail previously (Weiss et al., 2007). Intracellular fluorescence was quantified in a MACSQuant 10 analyser (Miltenyi, Bergisch-Gladbach, Germany) with the 638 nm laser and the 650 nm bandpass filter for pheophorbide A. Excluding debris, living cells were gated in the forward/sideward scatter and in each sample 30,000 cells were counted. BCRP inhibition by ketoconazole and DAK was
quantified by calculating the ratio between the median fluorescence with and without the inhibitor and by normalising the effects to those measured in the parental cell line. Possible quenching effects of the compounds were excluded because no effect on pheophorbide A fluorescence was observed for either compound in the parental cell line MDCKII.

2.7. OATP1B1 and OATP1B3 inhibition assay

HEK293 cells stably transfected with the organic anion transporter polypeptide 1B1 (OATP1B1) (HEK293-OATP1B1), OATP1B3 (HEK293-OATP1B3), or the empty control vector (HEK293-VC-G418) were used to assess OATP inhibition (König et al., 2000a, b). Cell lines were kindly provided by Dr. D. Keppler (German Cancer Research Centre, Heidelberg, Germany) and cultured under standard cell culture conditions with DMEM supplemented with 10% FCS, 2 mM glutamine, 100 µg/ml streptomycin sulphate, 100 U/ml penicillin, and 800 µg/ml G418. Inhibition of the OATPs was quantified by flow cytometry assessing the uptake of 8-FcA into HEK293 cells over-expressing the respective transporter and normalised to the control cell line as described previously (Weiss et al., 2013). Intracellular fluorescence was quantified in a MACSQuant 10 analyser with the 488 nm laser for excitation and the 525 nm bandfilter for emission. Living cells were gated in the forward/sideward scatter by using the unstained sample. For each concentration (0.1–100 µM), 30,000 cells were measured and each experiment was performed in biological triplicates or quadruplicates. Rifampicin was used as a control inhibitor. Possible quenching effects of the compounds were excluded because no effects on 8-FcA fluorescence by ketoconazole and DAK were observed in HEK293-VC-G418 cells.

2.8. Analysis of DAK concentrations in human plasma

For quantifying DAK concentrations in human plasma, we used plasma samples obtained in a previous drug-drug interaction trial (EudraCT number 2017-004453-16, ethical approval number AFmo-106/2018), in which 14 healthy volunteers received a single oral dose of ketoconazole (400 mg) (Rohr et al., 2021). Venous blood was drawn 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 3, 3.5, 4, 5, 7, 9, 12, 24, and 25 h after drug administration. Plasma was separated within 30 min after blood withdrawal and samples were stored at −25 °C until analysis. Plasma concentrations of DAK and ketoconazole were quantified by ultra-high-performance liquid chromatography coupled to triple quadrupole mass spectrometry (UPLC-MS/MS, Waters Xevo TQ-S and TQ-D, Waters, Eschborn, Germany). DAK and its metabolite were extracted from plasma by liquid-liquid extraction using tert-butyl methyl ether. To compensate analyte loss, each sample was spiked with ketoconazole-D₈. On both instruments, chromatographic separation was performed on an Acquity UPLC BEH C18 column (Waters, Eschborn, Germany) using a 5 min gradient program. The UPLC eluents consisted of acetonitrile plus 0.01% formic acid (eluent A) and water including 5% acetonitrile plus 0.01% formic acid (eluent B). All eluents were purchased from Biosolve (Valkenswaard, The Netherlands). Ketoconazole was quantified using mass-to-charge transitions of m/z 349.1→81.9 (ketoconazole) and m/z 539.5→81.9 (ketoconazole-D₈) in the TQ-D system. DAK was quantified using the Xevo-TQ S system monitoring mass-to-charge transitions of m/z 489.1→136.0 (DAK) and m/z 539.1→185.4 (ketoconazole-D₈). A linear calibration range was present at ketoconazole concentrations of 0.19 to 47 µM (0.1 to 25 µg/ml) and DAK concentrations of 0.2 to 205 nM (0.1 to 100 ng/ml). The lower limit of quantification was 0.2 µM for ketoconazole and 0.2 nM for DAK. Plasma concentrations were identified with an overall accuracy of 91.5–101% (ketoconazole) and 86.0–103% (DAK) and a precision of ≤ 6.5% (ketoconazole) and ≤ 13% (DAK). In an attempt to find other ketoconazole metabolites, a full scan analysis was performed using high-resolution mass spectrometry (Waters Xevo G2-XS QTof, Waters, Eschborn, Germany). Plasma samples taken 2 and 9 h after drug administration were scanned for [M + H]+ of m/z 531.1560 (ketoconazole), m/z 707.1881 (ketoconazole-glucuronide), 565.1615 (ketoconazole M₁), 563.1459 (ketoconazole M₂/M₄/M₂), 547.1510 (ketoconazole M₄-M₇), 545.1353 (ketoconazole M₁₀), 529.1404 (ketoconazole M₂₀), 507.1560 (ketoconazole M₂/M₂₅), 505.1404 (ketoconazole M₈), 503.1247 (ketoconazole M₁₈), 463.1298 (ketoconazole M₁₆), 420.0876 (ketoconazole M₁₂/M₁₃), and 257.0243 (ketoconazole M₉).

2.9. Statistical analysis

Data were analysed using GraphPad Prism Version 9.1.2 (GraphPad Software, San Diego, CA, USA). IC₅₀ values were calculated using the four-parameter fit (sigmoidal dose-response curves with variable slope).

3. Results

3.1. Inhibition of CYP2C19, CYP2D6, and CYP3A4 by DAK in comparison to ketoconazole

Ketoconazole is a well-known and potent CYP3A4 inhibitor. Our data for the first time demonstrate that its metabolite DAK also potently inhibits this enzyme in the upper nanomolar range, however with an about twofold lower potency compared to ketoconazole (Fig. 1, Table 1). CYP2C19 was inhibited by both compounds with a similar, but lower potency compared to CYP3A4 (Fig. 1, Table 1). Interestingly, CYP2D6 was inhibited about 10-times more potently by DAK compared to ketoconazole, which was only a moderate CYP2D6 inhibitor (Fig. 1, Table 1).

3.2. Inhibition of P-gp by DAK in comparison to ketoconazole

In the LLC cell system, DAK clearly increased intracellular calcein fluorescence in P-gp over-expressing MDRI cells, but not in the parental cell line LLC-PK1 indicating P-gp inhibition (Fig. 2A). However, IC₅₀ calculation was not possible due to missing plateau effects and therefore the f2 value was calculated instead as published previously (Weiss and Haefeli 2006) (Table 1). Ketoconazole increased calcein fluorescence in both cell lines (data not shown), indicating additional MRP inhibition. Quantification of P-gp inhibition was therefore not possible and P-gp inhibition was additionally tested in the P388 cell system. In this cell system, ketoconazole and DAK only increased intracellular calcein fluorescence in the P-gp over-expressing cell line P388/dx and not in the parental cell line P388 confirming P-gp inhibition (Fig. 2B, data only shown for DAK). The potency of ketoconazole was in the upper nanomolar range and was about 3-fold higher than that of DAK (Table 1).

3.3. Inhibition of BCRP by DAK in comparison to ketoconazole

Ketoconazole and DAK increased intracellular phosphorhodamine A fluorescence in MDCKII-BCRP but not in MDCKII cells clearly demonstrating moderate BCRP inhibition. For ketoconazole, calculation of an IC₅₀-value was not possible due to missing plateau effects (Fig. 3, Table 1).

3.4. Inhibition of OATP1B1 and OATP1B3 by DAK compared with ketoconazole

Ketoconazole and DAK decreased intracellular fluorescence of 8-FcA in the OATP1B1 and OATP1B3 over-expressing HEK293 cell lines, but not in the parental cell line demonstrating inhibition of OATP1B1 and OATP1B3 (Table 1, Fig. 4). Whereas the IC₅₀ value of ketoconazole was in the lower micromolar range, DAK was only a moderate inhibitor of these uptake transporters (IC₅₀ in the medium micromolar range).
3.5. Analysis of DAK concentrations in human plasma

In healthy volunteers receiving a single oral dose of 400 mg ketoconazole, plasma concentrations of ketoconazole within a dosing interval of 24 h ranged between 0.2 and 28 µM (0.1 and 14.9 µg/ml) and DAK concentrations ranged between 1.0 and 74 nM (0.5 and 36 ng/ml) (Fig. 5). The full scan experiments revealed that of all 12 metabolites studied, ketoconazole M23 was the only other metabolite detectable in plasma at an estimated concentration of only about 1% of the parent compound.

4. Discussion

Ketoconazole is a strong inhibitor particularly of CYP3A4 and P-gp and is therefore often used in drug-drug interactions studies investigating the influence of CYP3A4/P-gp inhibition on the pharmacokinetics of substrate drugs. A preliminary PBPK model for drug-drug interactions indicated that a ketoconazole metabolite might contribute to its perpetrator potential. In animals DAK is the major metabolite (Whitehouse et al., 1990, 1994a, b; Rodriguez and Acosta, 1997a), whereas in humans this has not been clarified yet. However, DAK is generated in human liver microsomes following Michaelis-Menten kinetics and elicits hepatocellular toxicity (Fukami et al., 2016) suggesting that it also plays an important role in humans. We, therefore, investigated, whether the ketoconazole metabolite DAK might contribute to the interaction potential of the mother compound, which comprises the inhibition of several CYPs and pharmacokinetically relevant drug transporters (P-gp, BCRP, OATP1B1 and OATP1B3) (Emoto et al., 2003; Vermeer et al., 2016).

Our data verified the very strong inhibition of CYP3A4 and P-gp by ketoconazole in the nanomolar range verifying its reputation as strong CYP3A4 and P-gp inhibitor. All other proteins investigated were also inhibited by ketoconazole, although with a lower potency (Table 1, Figs. 1-4). Our study is the first to demonstrate that also DAK inhibited all human CYPs and drug transporters investigated. However, the inhibitory potency was generally lower except for CYP2D6 and BCRP. Interestingly, CYP3A4 inhibition by DAK was only 2.4-fold and P-gp only 3.4-fold less potent than by ketoconazole, raising the question whether DAK might contribute to ketoconazole’s inhibitory potential in vivo.

According to very early publications on ketoconazole, its main metabolism in humans is attributed to microsomal hepatic enzymes and primarily consists of oxidation of the imidazole ring, degradation of the oxidized imidazole, oxidative O-dealkylation, oxidative degradation of the piperazine ring, and aromatic hydroxylation (Gascoigne et al., 1981; Heel et al., 1982; Hume and Kerkering 1983; Daneshmend and Warronck, 1988). However, these statements are not backed up with newer data or more advanced approaches and have been haunting the literature ever since, as already stated by others (Whitehouse et al., 1994a). Even more recent data “revisiting” ketoconazole metabolism did not clarify the main pathway, since only metabolites generated in vitro with human liver microsomes or hepatocytes were investigated (Fitch et al., 2009; Kim et al., 2017).

In rodents, DAK represents the main metabolite of ketoconazole, accumulates in the liver (Whitehouse et al., 1990, 1994a,b; Rodriguez & Aosta 1997a,b), and obviously contributes to inhibition of CYPs (Whitehouse et al., 1994a). This metabolite is also generated in vitro in human liver microsomes and hepatocytes by the enzyme arylacetamide deacetylase (AADAC) and further metabolised by flavin-containing monoxygenases (FMOs) to reactive metabolites (Fig. 6), which are held responsible for the hepatotoxicity of ketoconazole (Rodriguez et al., 1999; Rodriguez and Miranda 2000; Rodriguez and Buckholz 2005; Fukami et al., 2016; Foti and Dalvie 2016).

In our clinical trial, we for the very first time demonstrated that DAK is also formed in humans, although its maximum plasma concentrations are about three orders of magnitude lower than that of the parent compound.
compound. This agrees with an earlier trial that could not find DAK in plasma of patients on chronic ketoconazole therapy using a much less sensitive method with an LOQ of about 1 µg/ml (0.2 µM) (Badcock et al., 1987). The observed low concentrations could challenge the assumption that DAK is also the main metabolite in humans. However, previous animal studies did not measure plasma concentrations, which might also have been very low, but only examined liver extracts in which DAK was undoubtedly the major metabolite and reached concentrations in the lower millimolar range (Whitehouse et al., 1990a, 1994). Interestingly, when incubating human liver (HepG2) cells with ketoconazole, DAK could be measured only inside the cells and was not released into the supernatant (Wewering et al., 2017). Taken all these findings together, our study confirms that DAK is built also in humans but circulating concentrations are very low; nevertheless and in agreement with data in rodents DAK might be the main hepatic metabolite, which accumulates in the liver and is further metabolized or exported thus hardly reaching the systemic circulation. Assuming similar liver concentrations as in mice (millimolar), DAK might indeed contribute to the perpetrator characteristics of ketoconazole in drug-drug interactions or even be responsible for a substantial proportion of ketoconazole’s perpetrator action.

When searching for metabolites in human plasma after a single dose of 400 mg ketoconazole, our study revealed that apart from ketoconazole and DAK, ketoconazole M23 was the only detectable metabolite in plasma. However, the concentration was low, and so far, there are no data on whether this metabolite could be of clinical importance.

Limitations and open questions: (1) We did not clarify whether DAK is the main ketoconazole metabolite in humans, but we were able to...
demonstrate its presence in human plasma at nanomolar concentrations after oral administration and exclude relevant plasma concentrations of other metabolites. (2) Because the expression of the DAK-forming enzyme AADAC is not restricted to the liver but also occurs in the intestine (Probst et al., 1994; Kobayashi et al., 2012), DAK might also arise in enterocytes. However, it remains unclear whether this pathway contributes substantially to ketoconazole metabolism and whether DAK is also trapped in these cells as demonstrated for liver cells. (3) We only

Fig. 4. Concentration-dependent effect of ketoconazole and N-deacetyl ketoconazole on OATP1B1 and OATP1B3 mediated transport of 8-FcA in OATP1B1 (A) or OATP1B3 (B) overexpressing cells normalised to the mock-transfected control cell line. Each curve depicts the results of 3–4 experiments with 30,000 cells measured each. Data are expressed as mean ± S.E.M.

Fig. 5. Plasma concentration–time curve (mean with upper and lower limits) of ketoconazole and N-deacetyl ketoconazole (DAK) after 400 mg oral ketoconazole in 12 healthy volunteers.

Fig. 6. Proposed main metabolic pathway of ketoconazole in humans (according to Rodriguez and Acosta, 1997a; Fukami et al., 2016). AADAC, ary lacetamide deacetylase; FMO, flavin-containing monooxygenase.
investigated the inhibitory properties of the putative main metabolite DAK and not of other metabolites and cannot rule out that other metabolites might also contribute to the drug-drug interaction potential of ketoconazole. However, because no systemically relevant concentrations could be measured, this would also imply that high concentrations of these putative metabolites are only present in the liver and their contribution to an extraplastic interaction appears unlikely.

5. Conclusions

In conclusion, we have demonstrated that the putative major metabolite of ketoconazole, DAK, inhibits several CYPs and drug transporters, which might contribute to the perpetrator potential of ketoconazole. This, however assumes that DAK accumulates in human liver as demonstrated for rodents, because plasma concentrations after oral dosing were too low for relevant inhibition.

CRediT authorship contribution statement

Johanna Weiss: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration.

Kathrin Isabelle Foerster: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft.

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Jürgen Burchardt: Methodology, Validation, Resources, Writing – review & editing.

Gerd Mikus: Conceptualization, Resources, Writing – original draft.

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Walter Em Haefeli: Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision.

Conflict of interests

The authors declare no conflict of interests.

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