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Multiple Ascending Dose Pharmacokinetics of the new Antifungal BAL4815 after Intravenous and Oral Administration of its Prodrug BAL8557

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Revised Abstract

Background: BAL8557 is a water soluble pro-drug converted in plasma to the drug BAL4815. BAL4815 showed *in vitro* broad-spectrum antifungal activity against all major opportunistic fungi and true pathogenic fungi, including fluconazole-resistant strains. The pro-drug is particularly suited for intravenous (iv) and oral (po) administration. The objective of this study was to assess po and iv pharmacokinetics of the pro-drug and drug in humans, following repeated ascending doses.

Methods: In this double-blind, placebo controlled MAD study, we randomly assigned 32 healthy males into 4 cohorts (C) to receive either BAL8557 doses as BAL4815 equivalents (n=6/Cohort) or placebo (n=2/Cohort). Cohort 1 (C1) received an oral loading dose (LD) of 100 mg followed by once-daily maintenance doses (MD) of 50 mg up to Study Day 21. Cohort 2 (C2) an oral LD of 200 mg and MD of 100 mg. Cohort 3 (C3) received a 1 h-iv infusion LD of 100 mg followed by MD of 50 mg and Cohort 4 (C4) a 1 h-iv infusion LD of 200 mg and MD of 100 mg. Blood samples were collected up to 24 h after administration on selected study days and up to 480 h after the last administration. Pharmacokinetics were estimated by non-compartmental analysis.

Results: After *po administration,* 24-h AUC-values of BAL4815 reached 8.75 (C1) and 18.5 (C2) µg.h/mL and 21.6 (C1) and 40.3 (C2) µg.h/mL , on Study Days 1 and 21, respectively. After *i.v infusion*, 24-h AUC-values of BAL4815 were 7.31 (C3) and 12.9 (C4) µg.h/mL and 14.3 (C3) and 33.6 (C4) µg.h/mL, on Study Days 1 and 14, respectively. After both iv and po administrations a 4- to 5-fold accumulation of BAL4815 was observed in line with its elimination half-life (85 to 117 h). At steady-state, the systemic clearance was 2.4 to 4.1 L/h and the volume of distribution was 308 to 542 L.

24 h-urinary ratios of «6-β-hydroxycortisol/cortisol» were dose-, time- and treatment-independent.

Conclusion: After repeated po and iv administrations, the plasma levels of BAL4815 were dose proportional, as anticipated from single dose studies. There was no indication of inhibition or induction. Absolute bioavailability of the po formulation appeared excellent.

Introduction

The pro-drug BAL8557 was specifically designed as a water-soluble precursor suitable for intravenous infusion and oral administration.

In vitro, the pro-drug is rapidly catalyzed by plasma esterases in plasma of rat, monkey, and man with a half-life of less than one minute. Subsequently, the intermediate is converted to BAL4815 (active azole) and BAL8728 (pro-drug cleavage product) by an intra-molecular cleavage reaction.

The toxicity profile in animals was comparable to that of other azoles. Toxicological studies revealed no mutagenic, allergenic, phototoxic, or irritant potential. In animals, a very high relative bioavailability of the active azole was observed after intravenous and oral administration of the pro-drug.

The active azole is a potent inhibitor of ergosterol synthesis. *In vitro*, the active azole demonstrated broad-spectrum activity against all major opportunistic fungi, e.g., *Candida*, *Aspergillus*, *Cryptococcus*, and the true pathogenic fungi, including *Histoplasma capsulatum* and *Blastomyces dermatitidis*. The active azole also demonstrated strong antifungal activity against fluconazole-resistant *C. albicans*, and was active against *Zygomycetes, Absidia, Rhizopus*, and *Rhizomucor* species. In rat models, the active azole was highly effective against systemic candidiasis and aspergillosis.

Methods

The pharmacokinetics of active azole BAL4815 were investigated in a single center, double-blind, randomized, placebo-controlled, multiple dose study with two dose regimens for each of the two routes of administration (oral and intravenous). Thirty-two healthy male subjects were randomly assigned to four treatment groups to receive BAL8557 (six subjects per cohort) or placebo (2 subjects per cohort): Cohort 1 received an oral loading dose of 100 mg equivalents of BAL4815 in the form of pro-drug BAL8557 followed by a once-daily maintenance dose of 50 mg up to Study Day 21. Cohort 2 received an oral loading dose of 200 mg equivalents of BAL4815 followed by a once-daily maintenance dose of 100 mg up to Study Day 21. Cohort 3 received a constant rate 1 h-intravenous infusion of 100 mg as loading dose followed by a once-daily maintenance infusion of 50 mg up to Study Day 14. Cohort 4 received a constant rate 1 h-intravenous infusion of 200 mg as loading dose followed by a once-daily maintenance infusion of 100 mg up to Study Day 14.

Blood samples were collected up to 24 h after administration on selected study days at weekly intervals and up to 480 h after the last administration. Plasma concentrations of BAL4815 (active drug) and BAL8728 (pro-drug cleavage product) were quantified using a validated specific LC-MS/MS assay.

Pharmacokinetic parameters of BAL4815 and BAL8728 were estimated from their plasma concentrations by non-compartmental analysis using WinNonlin version 4.0.1. Urine was collected in 24-hour intervals on Study Day -1, Study Day 1, 8, 14, and at follow-up for the determination of ratios «6-β-hydroxycortisol/cortisol».

Results

Pharmacokinetics of BAL4815

Oral Administration (Cohorts 1 and 2): After oral administration, mean C_{max}-values were observed in plasma 2 to 3.5 hours after drug intake (Table 1, Figure 1). On Study Day 1, the mean AUC_{0-24h}-values of BAL4815 were 8.75 µg.h/mL (Cohort 1) and 18.5 µg.h/mL (Cohort 2). The mean AUC_{0-24h}-value seemed to increase fairly proportionally to the dose administered. On Study Day 21, AUC_{0-24h}-values were 21.6 µg.h/mL (Cohort 1) and 40.3 µg.h/mL (Cohort 2). Elimination half-lives of BAL4815 were long and could only reliably be determined after the last administration on Study Day 21 (Cohort 1: 98.4 h; Cohort 2: 84.5 h). At steady state (last study day), the volume of distribution was large and amounted to 346 L (Cohort 1) and 308 L (Cohort 2); systemic clearance was low and reached only 2.4 and 2.5 L/h for Cohort 1 and 2, respectively. The pharmacokinetic properties of BAL4815 in the present study were in agreement with those reported in a single ascending oral dose study [1].

Intravenous infusion (Cohorts 3 and 4): On Study Day 1, the mean AUC_{0-24h}-values of BAL4815 were 7.31 µg.h/mL (Cohort 3) and 12.9 µg.h/mL (Cohort 4) (Table 2, Figure 2). On Study Day 14, AUC_{0-24h}-values had increased to 14.3 µg.h/mL (Cohort 3) and 33.6 µg.h/mL (Cohort 4). Mean elimination half-lives of 93.0 and 117 hours were estimated on the last study day. At steady-state, systemic clearance (CL_{SS}) reached 4.1 to 3.2 L/h and the mean volume of distribution (V_{SS}) was 470 and 542 L, for Cohort 3 and 4, respectively. The pharmacokinetic properties of BAL4815 in the present study were in agreement with those reported in a single ascending intravenous dose study [2].

Inter-subject variability of C_{max} and AUC_{0-24h} of BAL4815 was low to moderate (10 - 42%) in all four cohorts.

Dose Proportionality: As confirmed by an one-way ANOVA, AUC and C_{max} of BAL4815 increased proportionally to the dose. Thus dose-proportionality was assumed for AUC and C_{max} for both iv and po routes of administration ($p \geq 0.09$). There was no significant effect of dose on CL_{SS} ($p \geq 0.36$), V_{SS} ($p \geq 0.58$) or $t_{1/2}$ ($p \geq 0.31$) which were estimated on the last study day.

Drug Accumulation: Drug accumulation 'R_{ACC}' of BAL4815 in plasma was estimated by «AUC_{0-24h} on the last study day divided by AUC_{0-24h} on Study Day 1» corrected for identical doses and assuming dose proportionality (Table 3). The thus estimated 4- to 5-fold accumulation is somewhat lower than the predicted drug accumulation of 5.6 to 7.5, calculated according to $R=1/1-e^{-k}t$, where t is the 24-h dosing interval and k the mean elimination rate constant assessed on the last study day (derived from mean elimination half-lives of 85 and 117 hours; cf Table 1 and 2). Obviously, steady state conditions have not been completely reached in all subjects after a dosing period of 2 weeks (iv) and possibly after dosing of 3 weeks (po).

Pharmacokinetics of Pro-drug Cleavage Product BAL8728

After oral administration only very few plasma samples had detectable plasma concentrations of BAL8728 and only up to 90 minutes after administration. A pharmacokinetic assessment was therefore, not possible.

After intravenous infusion, however, plasma concentrations of BAL8728 were quantifiable up to 8 hours after start of infusion. On Study Day 1, mean AUC_{last} -values reached 0.476 and 0.872 µg.h/mL. Mean elimination half-lives were short and amounted to approximately one hour in both dose groups. In agreement with its short half-life, no accumulation of BAL8728 was observed in plasma upon daily dosing of BAL8557. On the last day of administration, mean $AUC_{0-\infty}$-values of BAL8728 were less than 1% of the corresponding $AUC_{0-\infty}$-values of active azole BAL4815.

Ratio of «6-β-hydroxycortisol/cortisol»:

The 24-hour urinary excretion ratio of «6-β-hydroxycortisol/cortisol» is considered to be a useful non-invasive test to evaluate inducing or inhibiting properties of drugs, when subjects are their own control. While this test is well validated as a measurement of induction, its relevance is still under debate for certain forms of inhibition [3]. As shown in Table 4, there was no indication of inducing or inhibiting properties of BAL4815 over the course of the study.

Conclusions

BAL8557 was rapidly converted to its active form BAL4815. After repeated po and iv administrations, the plasma levels of BAL4815 behaved as anticipated from single dose studies, in a dose-proportional fashion without indication of an inhibition or induction. The absolute bioavailability of the oral formulation of BAL8557 appears to be excellent.

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Figure 1: *Mean plasma profiles of BAL4815 after once-daily oral administrations of BAL8557.*
Cohort 1: Loading dose of 100 mg equivalents of BAL4815 followed by once-daily doses of 50 mg equivalents of BAL4815.
Cohort 2: Loading dose of 200 mg equivalents of BAL4815 followed by once-daily doses of 100 mg equivalents of BAL4815.

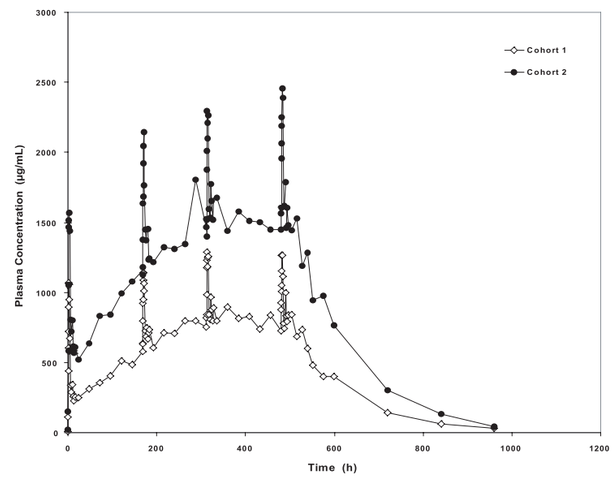

Figure 2: *Mean plasma profiles of BAL4815 after once-daily intravenous infusions of BAL8557 (infusion duration: 1 h).*
Cohort 3: Loading dose of 100 mg equivalents of BAL4815 followed by once-daily doses of 50 mg equivalents of BAL4815.
Cohort 4: Loading dose of 200 mg equivalents of BAL4815 followed by once-daily doses of 100 mg equivalents of BAL4815.

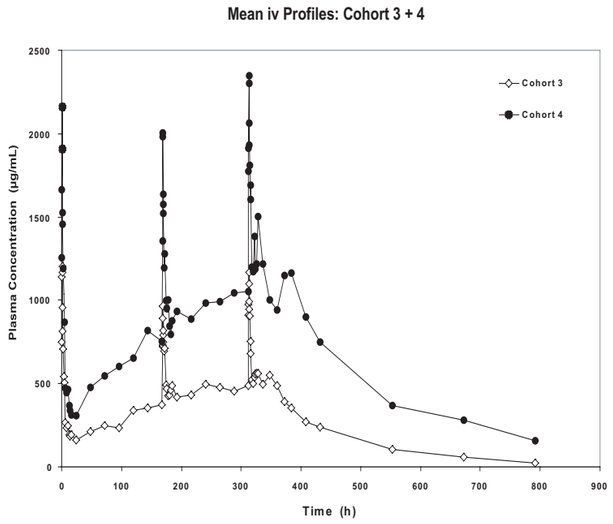

Table 1: *Pharmacokinetic parameter of BAL4815 estimated after once-daily oral administrations of BAL8557.*
Cohort 1: Loading dose of 100 mg equivalents of BAL4815 followed by once-daily doses of 50 mg equivalents of BAL4815.
Cohort 2: Loading dose of 200 mg equivalents of BAL4815 followed by once-daily doses of 100 mg equivalents of BAL4815.

	Study Day	Loading Dose (LD) Maintenance Dose (MD)	t_{max} (h)	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)	$AUC_{0-\infty}$ (µg.h/mL)	$t_{1/2}$ (h)	V_z/F (L)	CL_s/F (L/h)
Cohort 1	1	LD 100 mg	**2.0** (2.0 – 3.0)	**1.10** (± 0.173)	**8.75** (± 1.26)	-	**30.7** (± 14.8)	**214** (± 31.9)	**5.53** (± 1.92)
	8	MD 50 mg	**2.0** (1.5 – 3.0)	**1.20** (± 0.215)	**18.1** (± 4.45)	-	**51.0** (±13.7)*	**212** (± 92.9)*	**2.89** (± 0.595)
	14	MD 50 mg	**3.0** (1.2 – 4.0)	**1.40** (± 0.178)	**22.1** (± 4.92)	-	**82.1** (±43.2)*	**275** (± 161)*	**2.36** (± 0.479)
	21	MD 50 mg	**2.2** (1.5 – 3.0)	**1.37** (± 0.230)	**21.6** (± 4.30)	**130** (± 26.5)	**98.4** (± 21.3)	**346** (± 112)	**2.39** (± 0.430)
Cohort 2	1	LD 200 mg	**2.0** (1.2 – 3.1)	**1.85** (± 0.326)	**18.5** (± 3.11)	-	**31.0** (± 15.7)	**206** (± 45.4)	**5.28** (± 1.99)
	8	MD 100 mg	**3.0** (2.0 – 12)	**2.33** (± 0.472)	**34.0** (± 5.70)	-	**54.4** (±23.4)*	**234** (±79.4)*	**3.02** (± 0.543)
	14	MD 100 mg	**2.2** (1.5 – 4.0)	**2.61** (± 0.365)	**41.5** (± 6.02)	-	**68.9****	**250****	**2.45** (± 0.356)
	21	MD 100 mg	**3.5** (2.0 – 4.0)	**2.56** (± 0.434)	**40.3** (± 4.28)	**255** (± 80.5)	**84.5** (± 28.3)	**308** (± 118)	**2.51** (± 0.275)

Values are presented as arithmetic Mean ± SD of 6 subjects. Median and range are presented for t_{max}. V_z/F and CL_s/F are presented on Study Day 1, V_{SS}/F and CL_{SS}/F are presented on Study Day 8, 14, and 21.
**n=5; **n=2. Shaded values could not be reliably estimated*

Table 2: *Pharmacokinetic parameter of BAL4815 estimated after once-daily intravenous 1 h-constant rate infusion of BAL8557.*
Cohort 3: Loading dose of 100 mg equivalents of BAL4815 followed by once-daily doses of 50 mg equivalents of BAL4815.
Cohort 4: Loading dose of 200 mg equivalents of BAL4815 followed by once-daily doses of 100 mg equivalents of BAL4815.

	Study Day	Loading Dose (LD) Maintenance Dose (MD)	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)	$AUC_{0-\infty}$ (µg.h/mL)	$t_{1/2}$ (h)	V_z (L)	V_{SS} (L)	CL_s (L/h)
Cohort 3	1	LD 100 mg	**1.28** (± 0.122)	**7.32** (± 1.09)	-	**27.5** (± 15.2)	**269** (± 51.1)	**230** (± 49.0)	**8.01** (± 3.25)
	8	MD 50 mg	**0.987** (± 0.180)	**12.4** (± 3.77)	-	**42.6** (± 11.2)[a]	**226** (± 85.3)[a]	**275** (± 73.9)[a]	**4.45** (± 1.69)
	14[b]	MD 50 mg	**1.17** (± 0.237)	**14.3** (± 5.33)	**90.2** (± 52.2)	**93.0** (± 40.1)	**521** (± 205)	**470** (± 162)	**4.06** (± 1.97)
Cohort 4	1	LD 200 mg	**2.32** (± 0.445)	**12.9** (± 1.93)	-	**23.8** (± 5.30)	**298** (± 60.7)	**260** (± 54.2)	**8.85** (± 1.80)
	8	LD 100 mg	**2.52** (± 1.05)	**24.3** (± 5.79)	-	**13.7**[c]	**125**[c]	**126**[c]	**4.29** (± 1.00)
	14	LD 100 mg	**2.55** (± 0.883)	**33.6** (± 9.67)	**236** (± 31.5)	**117** (± 17.6)[d]	**514** (± 175)[d]	**542** (± 229)[d]	**3.19** (± 0.901)

Values are presented as arithmetic Mean ± SD of 6 subjects. CL_s is presented on Study Day 1, CL_{SS} is presented on Study Day 8 and 14.
[a]n=3; [b]n=5; [c]n=2; [d]n=4. Shaded values could not be reliably estimated

Table 3: *Drug accumulation of BAL4815 after once-daily oral or intravenous dosing*

	AUC_{0-24h}* on Study Day 1 (µg.h/mL)	**AUC_{0-24h} on last Study Day (µg.h/mL)**	**Accumulation Factor R_{ACC}**
Cohort 1 (po)	**8.75** (± 1.26)	**21.6** (± 4.30)	**4.9** (± 0.66)
Cohort 2 (po)	**18.5** (± 3.11)	**40.3** (± 4.28)	**4.5** (± 0.65)
Cohort 3 (iv)	**7.32** (± 1.09)	**14.3** (± 5.33)	**3.8** (± 1.0)
Cohort 4 (iv)	**12.9** (± 1.93)	**33.6** (± 9.67)	**5.2** (± 1.1)

Values are presented as arithmetic means ± SD;
**Values are corrected for dose (assuming dose proportionality)*

Table 4: *Mean (± SD) ratios of 24-hour urinary excretion of «6-β-hydroxycortisol/cortisol» after once-daily administration of BAL8557*

Group	**Oral Dose (Loading Dose) / Maintenance Dose**	**Ratio «6-β-hydroxycortisol/cortisol»**				
		Day -1	Day 1	Day 8	Day 14	Follow-up
Cohort 1 (po)	100 mg / 50 mg	**8.01** (4.39)	**8.80** (3.31)	**8.28** (2.52)	**7.77** (4.01)	**10.4** (5.94)
Cohort 2 (po)	200 mg / 100 mg	**8.87** (3.29)	**8.44** (2.18)	**5.95** (2.59)	**6.73** (2.71)	**6.89** (1.68)
Cohort 3 (iv)	100 mg / 50 mg	**11.7** (6.16)	**12.9** (2.24)	**10.1** (2.57)	**14.4**[a] (1.43)	**11.1**[a] (7.23)
Cohort 4 (iv)	200 mg / 100 mg	**5.81** (2.70)	**6.85** (3.11)	**6.52** (2.31)	**6.39** (4.37)	**4.96** (2.40)
Placebo[b]	-	**8.87** (2.81)	**10.0** (3.22)	**10.7** (7.49)	**9.31** (3.51)	**10.8** (5.04)

Values present Mean (± SD) of six subjects;
[a]=5; [b]=8