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**HAL Id: hal-02316685**

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# Evaluation of voriconazole anti-*Acanthamoeba polyphaga* *in vitro* activity, rat cornea penetration and efficacy against experimental rat *Acanthamoeba* keratitis

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Received 2 November 2017; returned 18 December 2017; revised 30 January 2018; accepted 26 February 2018

**Background:** *Acanthamoeba* keratitis (AK) is a sight-threatening infectious disease. Its effective and safe medical therapy remains highly debated. Recently, voriconazole, a monotriazole with noted *in vitro* activity against a large variety of fungi, has been successfully used both topically and systemically to treat human AK cases.

**Objectives:** To measure anti-*Acanthamoeba polyphaga* *in vitro* activity, anti-rat AK efficiency and rat cornea penetration of eye-drop and oral voriconazole.

**Methods:** *A. polyphaga* was maintained in axenic cultures. *In vitro*, amoebicidal and cysticidal activities of voriconazole were measured using an XTT assay. AK lesions of Sprague Dawley rats were scored from grade 0 to grade 3. For 21 days, from day 7 post-infection, voriconazole (1% solution) eye drops were instilled or voriconazole was administered by gavage (60 mg/kg/day). After killing, superficial corneal epithelium scrapings were cultured and analysed by PCR, and eye-globe histology was performed. Cornea and plasma concentrations were determined using 2D HPLC separation and tandem MS.

**Results:** *In vitro*, voriconazole inhibited trophozoite proliferation with an IC<sub>50</sub> value of 0.02 mg/L and an IC<sub>90</sub> value of 2.86 mg/L; no cysticidal effect was found. In AK rats, eye drops reduced clinical worsening from day 7 to day 14 post-infection and oral voriconazole was not effective. Voriconazole cornea concentrations were directly dependent on the frequency of eye-drop instillations, which resulted in lower plasma concentrations, whilst oral voriconazole resulted in lower cornea concentrations.

**Conclusions:** Present data underline the need for high-frequency eye-drop instillation regimens for efficient AK therapy.

## Introduction

Although its occurrence is low, human *Acanthamoeba* keratitis (AK) is a severe, sight-threatening condition, often associated with contact-lens wearing, with a substantial proportion of patients requiring further optical or therapeutic keratoplasty or enucleation. *Acanthamoeba castellanii* and *Acanthamoeba polyphaga* are the most common species to cause keratitis.<sup>1</sup> The efficacy and safety of currently available medical treatments are highly debated. Biguanides are commonly used as first-line treatment, with polyhexamethylene biguanide (PHMB) and chlorhexidine previously established as the most successful cysticidal agents *in vitro*.<sup>2</sup>

Recently, voriconazole, a monotriazole with noted *in vitro* activity against a large variety of fungi, has been successfully used both topically and systemically in human AK cases.<sup>3–5</sup> The aim of this study was to measure anti-*A. polyphaga* *in vitro* activity, anti-rat AK efficiency and rat cornea penetration of topical and oral voriconazole.

## Materials and methods

### *Acanthamoeba*

From axenic *A. polyphaga* cultures (isolate ATCC 50495, Rockville, MD, USA) grown at 30°C in 20 cm<sup>2</sup> flasks in Peptone Yeast Extract Glucose Broth (PYG)

**Table 1.** Rat instillation sequences of 1% voriconazole eye drops and voriconazole cornea concentration measured in the right non-infected cornea

Voriconazole eye-drop regimen		Voriconazole cornea concentration (last day of eye-drop sequences), mean ± SD
first instillation sequence (frequency/ duration)	subsequent instillation sequence (frequency/duration)	
hourly (13 times a day, 8 am to 8 pm)/ 3 days	none	5.6±4.4 ng/mg (5 rats) (day 10 p.i.)
hourly (13 times a day, 8 am to 8 pm)/ 3 days	every 2 h (7 times a day, 8 am to 8 pm)/ 11 days	2.38±1.6 ng/mg (5 rats) (day 17 p.i.)
hourly (13 times a day, 8 am to 8 pm)/ 3 days	every 2 h (7 times a day, 8 am to 8 pm)/11 days then every 4 h (4 times a day, 8 am to 8 pm)/ 7 days	0.32±0.15 ng/mg (9 rats) (day 28 p.i.)

p.i., post-infection.

medium, 80% trophozoite suspensions were obtained by refrigerating flasks in ice-water baths. To obtain encystment, washed trophozoites were resuspended in high salt encystment medium (250 mM NaCl, 4.6 mM MgSO<sub>4</sub> and 0.36 mM CaCl<sub>2</sub>) as previously described.<sup>6</sup>

**Voriconazole preparations**

Voriconazole solutions were prepared with sterile injection water using 10 mg/mL (1% w/v) intravenous voriconazole lyophilisate (Vfend, Pfizer, Paris, France) for *in vitro* studies and eye drops and 200 mg Vfend tablets for oral treatment.

**In vitro evaluation of voriconazole amoebicidal and cysticidal activities**

Trophozoites (10<sup>3</sup>/100 µL/well) were allowed to adhere to 96-well flat-bottom microplates (2 h, 37°C) and voriconazole was added for 72 h (final concentrations of 0.01, 0.05, 0.1, 0.5, 1, 2, 5, 20 and 40 mg/L). Trophozoite viability was measured using an XTT assay (Sigma-Aldrich, Saint-Quentin-Fallavier, France) (final concentration of 0.3 mg/mL, incubation for 6–24 h at 37°C). After subtracting the 630 nm absorbance value from corresponding 490 nm values, for wavelength correction of optical defects, results were expressed as the percentage of control (medium alone) culture well values. To evaluate cysticidal activity, voriconazole was added for 24 or 48 h (final concentrations of 5, 10, 50, 100 and 200 mg/L) in microplates containing 10<sup>3</sup> cysts/100 µL/well and maintained at 37°C for 5 days, i.e. until >80% trophozoite confluence in control wells. Trophozoite reversion was evaluated using XTT colorimetry after incubation for 2, 4 or 6 h at 37°C and absorbance reading and correction as above. In control wells, >90% trophozoite reversion was microscopically verified.

**Experimental A. polyphaga rat keratitis and voriconazole treatment**

All procedures were performed according to regulations of the French Ministry of Research after approval of the *ad hoc* Ethics Committee (No. 00755.02). In 150 g male Sprague Dawley specific-pathogen-free rats (Janvier, Le Genest-Saint-Isle, France), housed two per cage, AK was obtained as described.<sup>7</sup> Eyes were examined weekly using a slit lamp by the same experienced investigator and keratitis lesions were scored. The following grade scheme was used: grade 0, no corneal opacity; grade 1, corneal opacity visible only using oblique slit beam; grade 2, corneal opacity visible using retro-illumination but not sufficient to obscure iris details; or grade 3, corneal opacity visible using retro-illumination and obscuring iris details.<sup>8,9</sup> Nineteen rats with left eye AK received voriconazole (1% solution)

eye drops daily for 21 days in both eyes. Instillation sequences are summarized in Table 1. Orally treated AK rats received voriconazole by gavage (60 mg/kg/day, two daily doses).

**Ophthalmic microbiological analyses and histology**

On day 28 post-infection (p.i.), the rats were euthanized, blood samples were collected and superficial corneal epithelium scrapings were obtained for cultures (non-nutrient agar plates with *Escherichia coli* suspension overlay, examined for viable *Acanthamoeba* every week for 1 month). *Acanthamoeba* spp. quantitative PCR was performed in a Light Cycler 2.0 (Roche Diagnostics), using primers and probe as described by Qvarnstrom *et al.*<sup>10</sup> The reaction mix contained 1× FastStart DNA master mix (Roche Diagnostics, Meylan, France), 0.2 µM primers, 0.2 µM probe, 3.5 mM MgCl<sub>2</sub> and 10 µL of DNA in a total reaction volume of 25 µL. Cycling parameters were 10 min at 95°C followed by 45 cycles of 15 s at 95°C and 30 s at 63°C.

Eye globes of killed animals were punctured, placed in a 10% buffered formaldehyde solution and paraffin-embedded and 3 mm sections were stained with haematoxylin-eosin.

**Cornea and plasma voriconazole concentrations during topical and oral treatments in AK rats**

At the end of each instillation regimen as above, right (non-infected) cornea samples were thawed and weighed, and 100 µL of voriconazole-d5-surcharged methanol (internal standard) was added to each sample as an extraction/precipitation reagent. After vortex-mixing, tubes were incubated for 30 min at room temperature and sonicated for 15 min before centrifugation (16 000 g for 5 min). For plasma samples, each 50 µL aliquot was mixed with 100 µL of extraction/precipitation reagent, vortex-mixed and centrifuged in a like manner. Supernatants were transferred to HPLC vials and 1 µL was injected for quantification in a 2D HPLC separation and tandem MS system consisting of a 3200 QTRAP tandem mass spectrometer equipped with a TurboIonSpray source (AB Sciex, Framingham, USA). Sample clean-up was carried out online in a perfusion column preceding an analytical octadecyl column connected to the mass spectrometer. Quantification was performed by multiple reaction monitoring (MRM) in positive ion mode with voriconazole-d5 as the internal standard. The assay, conducted in accordance with US FDA bioanalytical guidelines,<sup>11</sup> resulted in lower and upper quantification thresholds of 0.005 and 0.01 µg/mL, respectively, for both cornea and plasma samples.

**Data analysis**

Statistics were performed using contingency analysis (Fisher’s exact tests). A P value <0.05 was considered significant.

**Table 2.** Evaluation of the clinical efficacy of eye-drop and oral voriconazole treatments against *A. polyphaga* rat keratitis symptoms

Group of rats	Grade 0 <sup>a</sup>	Grade 1 <sup>a</sup>	Grade 2 <sup>a</sup>	Grade 3 <sup>a</sup>	Grade worsening <sup>b</sup>
Untreated (n = 10)	1/10	1/10	4/10	4/10	9/10
Eye-drop voriconazole (n = 10)	0/10	4/10	5/10	1/10	1/10*
Oral voriconazole (n = 9)	0/9	1/9	5/9	3/9	4/9

<sup>a</sup>Grades were evaluated at day 14 p.i.<sup>b</sup>Ratios of animals with grade worsening from day 7 to day 14 p.i.\**P* < 0.01 compared with the untreated group.

## Results

### *In vitro* voriconazole amoebicidal activity

Optimal XTT staining to evaluate trophozoite viability was obtained after 24 h incubation (data not shown). In three triplicated independent experiments, voriconazole inhibited trophozoite proliferation in a concentration-dependent manner; incubation with 0.01, 0.05, 0.1, 0.5, 1, 2, 5, 20 and 40 mg/L voriconazole resulted in inhibition percentages of 44.4±1.3, 55.3±3.5, 67.9±22.5, 81.6±26.0, 77.5±10.3, 93.5±3.1, 98.6±1.4, 96.6±4.5 and 100±0.7 (mean ± SD), respectively, corresponding to an average IC<sub>50</sub> value of 0.02 mg/L and an average IC<sub>90</sub> value of 2.86 mg/L. After cyst incubation with 5, 10, 50, 100 and 200 mg/L voriconazole for 24 or 48 h, reversion to trophozoites was similar to that of untreated controls (>90%, *P* > 0.05).

### Voriconazole efficacy against *A. polyphaga* rat keratitis

As shown in Table 2, worsening of clinical symptoms from day 7 to day 14 p.i. was observed in fewer rats in the eye-drop group (topically treated with 1% voriconazole from day 7 to day 28 p.i.) than in the untreated control group (*P* = 0.001), and clinical infection similarly worsened in both groups from day 14 to day 28 p.i. (*P* > 0.05). In orally treated rats, worsening of clinical infection was similar to that of untreated controls from day 7 to day 28 p.i. (*P* > 0.05). Topical or oral voriconazole did not affect day 28 p.i. culture, PCR or histology positivities for *A. polyphaga* (Table S1, available as [Supplementary data](#) at JAC Online).

### Cornea and plasma voriconazole concentrations in AK rats treated with eye drops and oral voriconazole

As shown in Table 1, voriconazole cornea concentrations were directly dependent on the frequency of eye-drop instillations. Voriconazole cornea concentrations were higher after topical administration hourly and every 2 h than after administration every 4 h (*P* = 0.005, respectively). Following oral twice-a-day administration of 60 mg/kg/day for 21 days, mean cornea and plasma voriconazole concentrations averaged 1.72±1.79 ng/mg (as ng/μL cornea, considering that 90% of cornea tissue is composed of water)<sup>12</sup> and 3.29±2.27 mg/L in 10 rats (mean ± SD), respectively.

## Discussion

The voriconazole *Acanthamoeba* IC<sub>90</sub> evaluation (2.86 mg/L) in this study compares with previously reported values of 1–52.85 mg/L and strong inhibition observed over 40 mg/L.<sup>13,14</sup>

No cysticidal activity was detected, consistent with the reported cyst resistance of clinical isolates at 1 and 10 mg/L concentrations.<sup>15</sup> In other discrepant studies, however, cysticidal activity was obtained at 5–15 mg/L or at higher concentrations for collection strains and clinical isolates, respectively.<sup>16,17</sup> Differences in *Acanthamoeba* strains and methodologies (such as staining) may account for such variations.

In an AK rat model, topical and oral voriconazole doses were determined according to previous studies in humans.<sup>18</sup> Eye-drop treatment from day 7 p.i. reduced AK lesion worsening from day 7 to day 14 p.i. without later effects, whereas oral voriconazole therapy did not modify clinical lesions until day 28 p.i. In both untreated and treated infected eyes, *Acanthamoeba* persistence was established on day 28 p.i. by culture, PCR and histology.

For the first time, to our knowledge, corneal voriconazole concentrations were measured in non-infected eyes of voriconazole-treated AK rats to suitably document concentrations during infection. At the end of a 3-day hourly topical instillation sequence, cornea concentrations were in the same range as the estimated amoebicidal IC<sub>90</sub> and were markedly reduced after switching to instillations every 2 h. While using 1000 × more concentrated eye drops, resulting cornea concentrations were not proportionally increased compared with values obtained in normal and *Paecilomyces lilacinus* keratitis rabbits, respectively,<sup>19</sup> and appeared to depend more on instillation frequency than on voriconazole dose, consistent with an absence of the corneal ‘reservoir’ effect, as reported in human aqueous humour, and reminiscent of the need for high instillation frequency to maintain voriconazole corneal concentrations above the MIC<sub>90</sub> for most fungal species.<sup>20</sup> Accordingly, corneal voriconazole concentrations were found to be equivalent, after a regimen of eye drops every 2 h, to that in the aqueous humour of humans,<sup>20</sup> and elevated aqueous humour concentrations were rapidly obtained after eye instillation in humans.<sup>18</sup> In topically treated rats, plasma concentrations were close to those in healthy horses<sup>21</sup> and presumably lower than those associated with adverse side effects in humans.<sup>22</sup> Owing to ethical constraints, one eye of each rat was infected. Thus, voriconazole was quantified in non-infected cornea since infected corneas were used for microbiological/histological assessments. Since concentrations in infected corneas likely exceed those in non-infected ones, this may represent a limitation for extrapolating our results to infected corneas.<sup>12</sup>

Reminiscent of reports of aqueous humour in humans, in which voriconazole concentration was half of that in plasma 3 h after oral administration,<sup>23</sup> the oral regimen resulted in cornea concentrations



lower than the IC<sub>90</sub>. Unwanted side effects may, however, prevent the use of high systemic doses of voriconazole, which also exhibits high inter- and intra-individual variabilities in residual concentrations and non-linear pharmacokinetic characteristics.<sup>22</sup>

The present data seem to underline the need for high voriconazole corneal concentrations for AK therapy. Therefore, further studies are necessary to investigate other routes of administration to achieve higher corneal concentrations and to study the cysticidal effect of voriconazole on different *Acanthamoeba* strains and its efficacy in association with other drugs.

## Acknowledgements

We are grateful to Auda Alsafandi and Sarah Vazirnejad for technical support and for helpful discussion over the course of the *in vitro* study.

## Funding

Financial support was obtained from the University of Rouen, France.

R. R. is a postdoc fellow supported by the University of Rouen, France and the Normandie region, France.

## Transparency declarations

None to declare.

## Author contributions

J. G., L. L. G. and L. F. designed the study, J. G., L. L. G., P. C., S. L., E. C., C. A., F. D., A. F., R. R. and L. F. performed the experiments, J. G., L. L. G., M. M. and L. F. analysed and interpreted the results, J. G. and L. L. G. wrote the draft manuscript, J. J. B. revised the manuscript and M. M. and L. F. approved the final version of the manuscript.

## Supplementary data

Table S1 is available as [Supplementary data](#) at JAC Online.

## References

- Dart JK, Saw VP, Kilvington S. *Acanthamoeba* keratitis: diagnosis and treatment update 2009. *Am J Ophthalmol* 2009; **148**: 487–99.e2.
- Elder MJ, Kilvington S, Dart JK. A clinicopathologic study of *in vitro* sensitivity testing and *Acanthamoeba* keratitis. *Invest Ophthalmol Vis Sci* 1994; **35**: 1059–64.
- Cabello-Vilchez AM, Martín-Navarro CM, López-Arencibia A et al. Voriconazole as a first-line treatment against potentially pathogenic *Acanthamoeba* strains from Peru. *Parasitol Res* 2014; **113**: 755–9.
- Bang S, Edell E, Eghrari AO et al. Treatment with voriconazole in 3 eyes with resistant *Acanthamoeba* keratitis. *Am J Ophthalmol* 2010; **149**: 66–9.
- Tu EY, Joslin CE, Shoff ME. Successful treatment of chronic stromal *Acanthamoeba* keratitis with oral voriconazole monotherapy. *Cornea* 2010; **29**: 1066–8.
- Sykes DE, Band RN. Polyphenol oxidase produced during encystation of *Acanthamoeba castellanii*. *J Protozool* 1985; **32**: 512–7.
- Vasseneix C, Gargala G, François A et al. A keratitis rat model for evaluation of anti-*Acanthamoeba polyphaga* agents. *Cornea* 2006; **25**: 597–602.
- Polat ZA, Walochnik J, Obwaller A et al. Miltefosine and polyhexamethylene biguanide: a new drug combination for the treatment of *Acanthamoeba* keratitis. *Clin Exp Ophthalmol* 2014; **42**: 151–8.
- Ruddell TJ, Easty DL. Drug therapy in a murine model of *Acanthamoeba* keratitis. *Eye* 1995; **9**: 142–3.
- Qvarnstrom Y, Visvesvara GS, Sriram R et al. Multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*. *J Clin Microbiol* 2006; **44**: 3589–95.
- US Department of Health and Human Services, FDA. *Guidance for Industry, Bioanalytical Method Validation*. <http://www.gmp-compliance.org/guidemgr/files/4252FNL.PDF>.
- Cahane M, Ben SGJ, Barequet IS et al. Human corneal stromal tissue concentration after consecutive doses of topically applied 3.3% vancomycin. *Br J Ophthalmol* 2004; **88**: 22–4.
- Rocha-Cabrera P, Reyes-Batlle M, Martín-Navarro CM et al. Detection of *Acanthamoeba* on the ocular surface in a Spanish population using the Schirmer strip test: pathogenic potential, molecular classification and evaluation of the sensitivity to chlorhexidine and voriconazole of the isolated *Acanthamoeba* strains. *J Med Microbiol* 2015; **64**: 849–53.
- Schuster FL, Guglielmo BJ, Visvesvara GS. In-vitro activity of miltefosine and voriconazole on clinical isolates of free-living amebas: *Balamuthia mandrillaris*, *Acanthamoeba* spp., and *Naegleria fowleri*. *J Eukaryot Microbiol* 2006; **53**: 121–6.
- Sunada A, Kimura K, Nishi I et al. In vitro evaluations of topical agents to treat *Acanthamoeba* keratitis. *Ophthalmology* 2014; **121**: 2059–65.
- Martín-Navarro CM, López-Arencibia A, Arnalich-Montiel F et al. Evaluation of the *in vitro* activity of commercially available moxifloxacin and voriconazole eye-drops against clinical strains of *Acanthamoeba*. *Graefes Arch Clin Exp Ophthalmol* 2013; **251**: 2111–7.
- Iovieno A, Miller D, Ledee DR et al. Cysticidal activity of antifungals against different genotypes of *Acanthamoeba*. *Antimicrob Agents Chemother* 2014; **58**: 5626–8.
- Neoh CF, Leung L, Chan E et al. Open-label study of absorption and clearance of 1% voriconazole eye drops. *Antimicrob Agents Chemother* 2016; **60**: 6896–8.
- Sponsel W, Chen N, Dang D et al. Topical voriconazole as a novel treatment for fungal keratitis. *Antimicrob Agents Chemother* 2006; **50**: 262–8.
- Vemulakonda GA, Hariprasad SM, Mieler WF et al. Aqueous and vitreous concentrations following topical administration of 1% voriconazole in humans. *Arch Ophthalmol* 2008; **126**: 18–22.
- Clode AB, Davis JL, Salmon J et al. Evaluation of concentration of voriconazole in aqueous humor after topical and oral administration in horses. *Am J Vet Res* 2006; **67**: 296–301.
- Dolton MJ, Mikus G, Weiss J et al. Understanding variability with voriconazole using a population pharmacokinetic approach: implications for optimal dosing. *J Antimicrob Chemother* 2014; **69**: 1633–41.
- Hariprasad SM, Mieler WF, Holz ER et al. Determination of vitreous, aqueous, and plasma concentration of orally administered voriconazole in humans. *Arch Ophthalmol* 2004; **122**: 42–7.