Case report

Use of tafenoquine to treat a patient with relapsing babesiosis with clinical and molecular evidence of resistance to azithromycin and atovaquone

Luis A. Marcos, Annie Leung, Laura Kirkman, Gary P. Wormser

Tafenoquine is a highly effective treatment for Babesia microti infections in animal models. An immunocompromised patient infected by a strain of B. microti that was at least partially resistant to both azithromycin and atovaquone was treated with tafenoquine. Systematic clinical studies using tafenoquine for treating other patients with babesiosis should be considered.

Tafenoquine is an 8-aminoquinoline primaquine analogue that received United States Food and Drug Administration approval in 2018 for two indications: prophylaxis of malaria for up to 6 months in total duration and prevention of relapse of Plasmodium vivax malaria [1,2]. Because of the long half-life of the drug of approximately 14–17 days in humans, a single dose of the drug can be administered once per week to prevent malaria [1–5].

Experimental data from 3 different studies conducted using hamsters or mice [6–8], including highly immunocompromised mice (severe combined immunodeficiency [SCID] mice [7]), have demonstrated that tafenoquine can rapidly clear Babesia microti parasites. Therefore, this drug may have a potential role in the treatment of patients with babesiosis, particularly for patients who are highly immunocompromised, such as those who have been treated with the drug rituximab [9], who can require many months of antiparasitic drug therapy before a cure is achieved [10–12].

To begin to understand the potential therapeutic role for tafenoquine, an immunocompromised adult patient with multiple relapses of a B. microti infection was eventually treated with a 6 week course of tafenoquine alone. Prior to starting tafenoquine, the B. microti parasite causing this patient’s infection was found to be at least partially resistant to both azithromycin and atovaquone. Tafenoquine was well tolerated and over the course of the nearly 19 months of follow-up since completion of the 6 week drug regimen of tafenoquine the patient has remained well.

Case summary

A 36 year old male was hospitalized on January 9, 2019 because of unexplained fevers for approximately 2 weeks. He was diagnosed with babesiosis based on a positive blood smear (8.5%). His nadir hemoglobin level was 5.7 g/dL, and he was transfused 3 units of blood. He had a history of granulomatosis with polyangiitis diagnosed in 2001. He had been remotely treated with methotrexate and cyclophosphamide, and he had received two doses of rituximab, the last of which was administered in January of 2017. Since the patient was only being treated with 7.5–20 mg of prednisone per day at the time of hospitalization, he was not regarded as being immunocompromised. The dose of prednisone was 7.5 mg during the first hospitalization, which he continued to receive during the first course of treatment for babesiosis.

The patient's initial treatment for B. microti infection was with a combination of atovaquone and azithromycin for 10 days (Table 1) with symptom resolution. However, the dosage of atovaquone was lower than usual [13]. Blood smears on both 1/11/19 and 3/6/19 were...
Table 1: Summary of Babesiosis Treatment Courses (all oral except where indicated; January 2019-April 2020).

| Initiation date and reason for treating with antiparasitic drug therapy | Azithromycin (dose) | Chloroquine (dose) | Tafenoquine (dose) |
|------------------------------------------------------------------------|---------------------|--------------------|------------------|
| 3/29/19 for 84 days due to fever, night sweats, chills and myalgias. | 750 mg QD | 750 mg QD | 750 mg QD |
| 8/28/19 for 20 days due to recurrent fever. | 750 mg QD | 750 mg QD | 750 mg QD |
| 9/17/19 for 14 days due to persistent positive blood smear. | 500 mg QD | 750 mg QD | 750 mg QD |
| 10/1/19 for 45 days due to continuing positive blood smear. | 500 mg QD | 750 mg QD | 750 mg QD |
| 11/14/19 for 14 days due to recurrent fever. | 500 mg QD | 750 mg QD | 750 mg QD |
| 12/17/19 for 4 days due to night sweats and fatigue. | 1000 mg QD | 750 mg QD | 750 mg QD |

Prior to initiation of tafenoquine the patient tested negative for glucose-6-phosphate dehydrogenase deficiency. An EKG showed a QTc interval of 409 ms; repeated electrocardiograms while taking tafenoquine did not show any QT interval changes. The patient tolerated the tafenoquine drug regimen well with no adverse effects. The patient has remained asymptomatic through 11/9/21 and is considered cured.

Resistance testing: methods

*Babesia microti* DNA was isolated from a blood sample collected on December 18, 2019 (parasitemia 0.08%) from the patient described. Nucleic acid amplification and direct Sanger DNA sequencing of the *cytb* and *rpl4* genes were performed using methods described previously [14]. Point mutations were found in each gene...
leading to amino acid changes previously correlated with treatment failure, CYTb (Y272C) and RPL4 (R86C) [15]. Cytochrome b (CYTb) is a highly conserved mitochondrial protein and a well described target of atovaquone in apicomplexan parasites including B. microti [14,15]. The mutation at position 272 is at a highly conserved region of the CYTb ubiquinol binding pocket. Likewise, mutations in RPL4 have been described in relapsed disease in patients who have been treated with azithromycin, and position 86 is highly conserved with alternative amino acid changes being reported at this site [14,15]. No testing was available to determine if the strain of B. microti was resistant to clindamycin. As there is no in vitro system for testing the inhibitory effects of drugs against B. microti, there is no way to directly measure a shift in drug sensitivity. Some patients with reported resistance mutations were eventually successfully treated with atovaquone and azithromycin, albeit at higher than usual doses [12,14]; therefore, the clinical relevance of these mutations needs to be more extensively studied.

Discussion

The patient described in this report experienced multiple relapses of B. microti infection and was eventually found to be infected with a B. microti strain regarded as at least partially resistant to both azithromycin and atovaquone [12,13]. His relapsing clinical course was likely because he was still immunosuppressed from the prior treatment with rituximab, in conjunction with an initial anti-babesia drug regimen of just 10 days duration using azithromycin combined with a lower than usual dose of atovaquone (Table 1); a 10-day treatment regimen is primarily intended for treating non-immunocompromised patients [13].

Although the patient was only receiving low dose prednisone at the time he became ill (7.5 mg/day which was later transiently increased to 20 mg/day when he experienced exacerbations of symptoms of his underlying autoimmune disease), he had received a dose of rituximab approximately 2 years before he was diagnosed with babesiosis. Consistent with the long-term effects of rituximab, the patient was found to be seronegative for antibodies to B. microti as late as 11/9/19 [10,11]. On 1/29/20 he was begun on a malonate®-based treatment regimen that included high doses of azithromycin and atovaquone, plus clindamycin (Table 1), malonate®-based treatment regimens have been previously used successfully to treat babesiosis patients, who had relapsed despite other drug regimens [10,12]. The patient’s last positive babesia blood smear was found on 1/30/20 and the last positive PCR test on 2/28/20.

After excluding that the patient had glucose-6-phosphate dehydrogenase deficiency [14,16], he was additionally treated with tafenoquine as a single agent starting on 3/10/20 for 6 weeks. He received 200 mg per day for 3 consecutive days followed by 200 mg once per week. Blood smears in 2020 performed on 3/23, 3/31, 4/8, 4/20, and 5/22 were all negative, as was PCR testing performed on 3/23, 3/31, 4/20, and 5/22. As of November 9, 2021, the patient has remained completely well. Given that patient became seropositive for antibodies to B. microti by 1/28/20, arguably indicating that the effects of the rituximab on the humoral immune system had largely disappeared, and that he was prescribed an intensive malonate®-based treatment regimen after documentation of seroconversion, it is impossible to conclude that the drug tafenoquine provided any clinical benefit. What can be stated is that it was well tolerated, with, as expected, no QTc prolongation on serial electrocardiograms [17]. It should be emphasized that a well-tolerated, single-drug treatment regimen, administered on a once per week basis, is unprecedented in the management of patients with babesiosis. Therefore, this single drug regimen may be of potential clinical importance, especially for treating highly immunocompromised patients with babesiosis, who require a minimum of at least 6 weeks of treatment, often extending into many months [10,13]. Systematic clinical studies using tafenoquine for treating patients with B. microti infections should be considered.

Declaration Section

Funding

None.

Conflict of interest

Dr. Marcos does not have any disclosures. Dr. Kirkman does not have any disclosures. Annie Leung does not have any disclosures. Dr. Wormser reports receiving research grants from the Institute for Systems Biology and Pfizer, Inc. He has been an expert witness in malpractice cases involving babesiosis; and is an unpaid board member of the non-profit American Lyme Disease Foundation.

Ethics approval

Stony Brook University IRB approval # 1210472 “Biomarkers for diagnosis and prognosis for Babesia”. Label-off use of tafenoquine for babesiosis in this case was a shared-decision between the treating physician and the patient as salvage therapy.

CRediT authorship contribution statement

Luis Marcos: Conceptualization, Writing – review & editing. Gary Wormser: Conceptualization, Writing – review & editing. Laura Kirkman: Susceptibility testing. Writing – review & editing. Annie Leung: Susceptibility testing, Writing – review & editing.

Consent to participate

Informed consent was obtained for study IRB #1210472.

Consent for publication

Patient consented for publication for this brief report.

References

[1] Tafenoquine (Arakoda; Krintafel) for malaria, Med Lett, 61, 2019, pp. 101–104.
[2] Chen V, Daily JP. Tafenoquine: the new kid on the block. Curr Opin Infect Dis 2019;32:607–12.
[3] Berman JD. Approval of Tafenoquine for malaria chemoprophylaxis. Am J Trop Med Hyg 2019;100:1301–4.
[4] Kaufman MB. Pharmaceutical approval update. P T 2018;43:659–61.
[5] Brueckner RP, Lasseter K, Lin ET, Schuster BG. First-time-in-humans safety and pharmacokinetics of WR 238605, a new antimalarial. Am J Trop Med Hyg 1998;58:645–9.
[6] Marley SE, Eberhard ML, Steurer FJ, Ellis WL, McGreevy PB, Rubeush 2nd TK. Evaluation of selected antiprotozoal drugs in the Babesia microti-hamster model. Antimicrob Agents Chemother 1997;41:91–4.
[7] Mordue DG, Wormser GP. Could the drug Tafenoquine revolutionize treatment of Babesia microti infection? J Infect Dis 2019;20:442–7.
[8] Carvalho UM, Tuvinhutuga B, Nagraha AB, Sivakumar T, Yokokura N. Activities of artesunate-based combinations and tafenoquine against Babesia bovis in vivo and Babesia microti in vitro. Parasites Vectors 2020;13:362. https://doi.org/10.1186/s12977-020-04235-7
[9] Gkrania-Klotsas E, Kamaratnate DS. Serious infectious complications after rituximab therapy in patients with autoimmunity: Is this the final word? Clin Infect Dis 2021;72:738–42.
[10] Raffals J, Wormser GP. Persistence of babesiosis for > 2 years in a patient on rituximab for rheumatoid arthritis. Diagn Microbiol Infect Dis 2016;85:231–2.
[11] Krause PJ, Gbewurz BE, Hill D, Marty FM, Vannier E, Foppa IM, et al. Persistent and relapsing babesiosis in immunocompromised patients. Clin Infect Dis 2008;46:370–6.
[12] Wormser GP, Prasad A, Neuhaus E, Joshi S, Nowakowski J, Nelson J, et al. Emergence of resistance to azithromycin-atovaquone in immunocompromised patients with Babesia microti infection. Clin Infect Dis 2010;50:381–6.
[13] Krause PJ, Auswaert PC, Banuru RR, Branda JA, Falk-Vitter YT, Lantos PM, et al. Clinical practice guidelines by the Infectious Diseases Society of America (IDSA):
2020 guideline on diagnosis and management of babesiosis. Clin Infect Dis 2021;72:183–9.

[14] Simon MS, Westblade LF, Dziedziech A, Visone JE, Furman RR, Jenkins SG, et al. Clinical and molecular evidence of atovaquone and azithromycin resistance in relapsed Babesia microti infection associated with rituximab and chronic lymphocytic leukemia. Clin Infect Dis 2017;65:1222–3.

[15] Lemieux JE, Tran AD, Freimark L, Schaffner SF, Goethert H, Andersen KG, et al. A global map of genetic diversity in Babesia microti reveals strong population structure and identifies variants associated with clinical relapse. Nat Microbiol 2016;1(7):16079. https://doi.org/10.1038/nmicrobiol.2016.79

[16] White NJ. Tafenoquine— a radical improvement? N Engl J Med 2019;380:285–6.

[17] Green JA, Patel AK, Patel BR, Hussaini A, Harrell EJ, McDonald MJ, et al. Tafenoquine at therapeutic concentrations does not prolong Fridericia-corrected QT interval in healthy subjects. J Clin Pharmacol 2014;54:995–1005.