Journal of Antivirals & Antiretrovirals

Bosseboeuf et al., J Antivir Antiretrovir 2018, 10:1
DOI: 10.4172/1948-5964.1000173

Research Article Open Access

Azithromycin Inhibits the Replication of Zika Virus

Eve Bosseboeuf[1], Maite Aubry[1,2], Tuxuan Nhan[1,2], Jean Jacques de Pina[1,2], Jean Marc Rolain[3], Didier Raoult[3] and Didier Musso[1,2*]

[1]Pôle de recherche et de veille sur les maladies infectieuses émergentes, Institut Louis Malardé, BP 30, 98713, Tahiti, Polynésie française

[2]IRD, AP-HM, SSA, VITROME, IHU-Méditerranée Infection, Aix Marseille Université, France

[3]IRD, APHM, MEPHI, IHU Méditerranée Infection, Faculté de Médecine et de Pharmacie, Aix Marseille Université, 19-21 boulevard Jean Moulin, 13385 Marseille CEDEX 05, France

[*]**Corresponding author:** Didier Musso, Pôle de recherche et de veille sur les maladies infectieuses émergentes, Institut Louis Malardé, Po Box 30, 98713, Tahiti, Polynésie française, Tel: 689.40.416.470; E-mail: dmusso@ilm.pf

Received date: February 18, 2018; **Accepted date:** March 13, 2018; **Published date:** March 19, 2018

Abstract

Background: The emergence of Zika virus (ZIKV) is associated to dramatic complications in fetuses and neonates. As there is no vaccine and no drug to prevent and treat ZIKV infections, there is an urgent need to have active drugs against ZIKV that can be used during pregnancy. Large screening strategies suggested that azithromycin (AZ) has an *in vitro* activity against ZIKV, we provide additional data supporting this hypothesis.

Methods: We tested the efficacy of AZ on ZIKV-infected Vero cells at a concentration that can be reached *in vivo* in amniotic fluid. We conducted two experiments with addition to infected cells of a single dose or multi doses of 50 mg/L of AZ, and analyzed ZIKV replication by immunofluorescence assay (IFA) and by measuring viral RNA loads at different times up to 96 h post-infection (hpi).

Results: Addition of a single dose of 50 mg/L of AZ prevented replication of ZIKV during 48 hpi; after 48 hpi, ZIKV replication was detected by IFA but viral RNA loads remained lower than in untreated infected cells. ZIKV replication was inhibited by addition of multi doses of 50 mg/L of AZ.

Conclusions: Our data confirm the *in vitro* activity of AZ against ZIKV. Since there will be no active specific drugs and vaccine available soon against ZIKV, AZ might be the first compound that could prevent and treat ZIKV infections, with the advantages of being an approved and safe drug usable during pregnancy.

Keywords: Zika virus; ZIKV; Antibiotics; Antiviral; Azithromycin; Emergence; Pregnancy

Introduction

Zika virus (ZIKV) is an arbovirus belonging to the *Flavivirus* genus in the *Flaviviridae* family. ZIKV was first described in 1947 and less than 20 human infections have been reported over the next 60 years. The first ZIKV outbreaks were reported in the Pacific in Yap (Federated States of Micronesia) in 2007 and in French Polynesia in 2013-2014. During the French Polynesian ZIKV outbreak, severe complications (Guillain-Barré syndromes) and non-vector borne transmission of the virus (maternofetal, sexual and post-transfusion) were described for the first time. Subsequently, ZIKV spread in other Pacific Islands and in the Americas causing serious birth defects [1,2].

Currently there is no vaccine and no drug approved to prevent and to treat ZIKV infections [3-5]. Using large screening strategies, several compounds have been found to have an *in vitro* activity against ZIKV including anticancer, immunosuppressant, antiviral, antiparasitic, antibiotics, antirheumatic, statin, iron chelator, antihemetic, antidepressant, and antifungal drugs [3,6-10]. However, most of these drugs cannot be used during pregnancy, their safety during pregnancy has not been demonstrated, and/or the effective concentration is higher than the maximum plasma concentration [6]

In vitro efficacy of several antibiotics against arboviruses has been reported [6] but to our knowledge, data on the efficacy of antibiotics to limit the growth of ZIKV are limited to preliminary results with daptomycin (lipopeptid), azithromycin (AZ) (macrolide) and kitasamycin (macrolide) [6-8,11,12].

As the main at-risk population for ZIKV infection is pregnant women, we investigated the efficacy of AZ, a safe antibiotic usable during pregnancy [13] to inhibit the replication of ZIKV *in vitro*. We characterized the anti-ZIKV properties of AZ in African green monkey kidney cells (Vero), a model widely used to study viral infections. We tested the efficacy of AZ on ZIKV-infected Vero cells at a concentration that can be reached *in vivo* in amniotic fluid after a single oral dose of 1 g of AZ [14] We provide additional data to those recently published by using a different timing of infection and by testing the *in vitro* efficacy of a single dose versus multi doses of AZ on ZIKV-infected cells [7].

Material and Methods

All experiments were performed in duplicate.

Virus, cell culture and antibiotic compound

ZIKV strain PF13/251013-18 was isolated from the serum of a French Polynesian patient in 2013 and was propagated in Vero cells (ATCC, Manassas, VA) as previously reported [15,16].

Vero cells were grown at 37°C in an atmosphere of 5% CO_2 in minimum essential medium supplemented with 2% fetal bovine serum (FBS) and 3% sodium bicarbonate 7.5% (Life Technologies, Carlsbad, CA).

AZ dihydrat (Sigma-Aldrich, France) was diluted at final concentration of 50 mg/L in the culture medium described above. This concentration corresponds to those detected in amniotic fluid 24 h after oral administration of a single dose of 1 g of AZ (36 ± 27 mg/L) [14].

Cell viability assays

Cell viability was tested using the tetrazolium reduction assay as recommended [17]. Different experiments were conducted using Vero cells uninfected with ZIKV and untreated with AZ as control; cells infected with ZIKV but non-treated with AZ; cells infected with ZIKV and treated with single or multi doses of AZ; and cells uninfected with ZIKV but treated with single or multi doses of AZ.

For all experiments, 1.6×10^4 Vero cells were added to each well of 96-well plates and incubated overnight at 37°C in 5% CO_2. In experiments using uninfected cells, 100 µL of culture medium with or without 50 mg/L of AZ were added to all wells. In experiments using ZIKV-infected cells, 100 µL of ZIKV diluted in culture medium was inoculated on cells of each well at a multiplicity of infection (MOI) of 0.1; inoculated cells were incubated one hour at 37°C in 5% CO_2, and then inoculum were removed and replaced by 100 µL of culture medium with or without 50 mg/L of AZ. In experiments including several doses of AZ, the medium was supplemented every 24 h with 50 mg/L of AZ. For all experiments, the medium was removed from 8 wells immediately and then 24, 48, 72 and 96 h after being added. Each well was washed twice with 400 µL of PBS and 50 µL of thiazolyl blue tetrazolium bromide (MTT, Sigma-Aldrich, France) were added. Plates were incubated for 45 m at 37°C in 5% CO_2, and then MTT was removed and replaced by 100 µL of dimethyl sulfoxide (DMSO). Plates were incubated for 10 m in the dark at room temperature. Presence of viable cells was visualized by the development of purple color. The suspension was transferred to cuvettes of a spectrophotometer and the optical density (OD) was read at 550 nm by using DMSO as a blank. The percentage of viable cells was determined as follows: cell viability (%)=Mean OD/Control OD × 100%. For each experiment, results were expressed as a mean of values of viable cells obtained in 8 wells in duplicate.

AZ activity on ZIKV-infected cells testing

In 24-well plates, 2.5×10^5 Vero cells/well were added and incubated overnight at 37°C in 5% CO_2. Cells were inoculated with 1 mL of ZIKV diluted in culture medium at a MOI of 0.1. After one hour of incubation at 37°C in 5% CO_2, inoculum were removed and replaced by 1 mL of culture medium containing either no AZ (control) or 50 mg/L of AZ.

Two types of experiments were then performed using single or multi doses of AZ. In the experiments including a single dose of AZ, cell culture supernatants (~1 mL) were retrieved from 3 wells immediately and then 24, 48, 72 and 96 h after the addition of AZ. In the multi doses experiments, cell culture supernatants were supplemented every 24 h with 50 mg/L of AZ; cell culture supernatants (~1 mL) were retrieved from 3 wells immediately after the first addition of AZ and then every 24 h during 96 h.

All cell culture supernatant samples were supplemented with 20% FBS and stored at -80°C until analysis of ZIKV RNA replication using real-time reverse transcription-polymerase chain reaction (RT-PCR). Moreover, the cells from 24-well plates were fixed on a glass slide and tested for the presence of replicative ZIKV by immunofluorescence assay (IFA). For each experiment, results were expressed as a mean of data obtained by RT-PCR or by IFA in 3 wells in duplicate.

ZIKV RNA quantification

ZIKV RNA loads were determined as previously described [16]. Briefly, viral RNA was extracted from 200 µL of each cell culture supernatant sample using the QIAcube extraction system (Qiagen, Canada), and quantified by real-time RT-PCR using a ZIKV RNA transcript serially diluted to generate a standard curve. ZIKV RNA loads were expressed in $\log_{10}$ copies/mL.

Hours	Control	Infected untreated cells	Infected cells with AZ single dose	Infected cells with AZ multi doses	Uninfected cells with AZ single dose	Uninfected cells with AZ multi doses
0	100 ± 0	142 ± 34	116 ± 12	116 ± 9	113 ± 13	107 ± 15
24	150 ± 5	142 ± 2	95 ± 19	88 ± 19	83 ± 22	85 ± 25
48	119 ± 15	94 ± 11	54 ± 14	24 ± 11	29 ± 15	20 ± 10
72	84 ± 1	78 ± 16	38 ± 23	16 ± 14	8 ± 7	6 ± 6
96	42 ± 14	34 ± 16	9 ± 4	3 ± 3	3 ± 3	3 ± 3

Table 1: Cell viability assay. Cell viability was measured at 0, 24, 48, 72 and 96 h for cells non-infected with ZIKV and non-treated with AZ (control); cells infected with ZIKV but non-treated with AZ; cells infected with ZIKV and treated with 50 mg/L of AZ in single dose or multi doses; and cells non-infected with ZIKV but treated with 50 mg/L of AZ in single dose or multi doses. Results are expressed in % of viable cells compared to the Control (mean and standard deviation). 100% is for control immediately after infection.

Detection of replicative ZIKV by IFA

Detection by IFA of replicative ZIKV in Vero cells was performed as previously described [16], using anti-flavivirus mouse antibodies 4G2 (Absolute Antibody, Oxford, United Kingdom) and goat anti-mouse IgG secondary antibody FITC-conjugate (LifeTechnologies, Carlsbad, CA) both diluted at 1:50. A green fluorescent coloration indicated the presence of replicative ZIKV in cells.

Results

Cell viability assays

In the control experiment (cells non infected with ZIKV and non-treated with AZ), the mean percentage of viable cells increased from 100% to 150 ± 5% during the first 24 h, and then decreased steadily until 42 ± 14 % at 96 h (Table 1 and Figure 1). Compared to the control, the mean percentages of viable cells after infection with ZIKV but without treatment with AZ were slightly lower at all-time points from 24 h post-infection (hpi), suggesting minor effect of the virus on cell viability. In contrast, after treatment with single or multi doses of AZ at 50 mg/L, the mean percentage of viable cells previously infected or uninfected with ZIKV dramatically decreased from 0 to 96 h post-treatment (range: 107 ± 15%-116 ± 12%/116 ± 9% and 3 ± 3%-9 ± 4%, respectively). These results suggest that AZ has a toxic effect on Vero cells at the concentration of 50 mg/L.

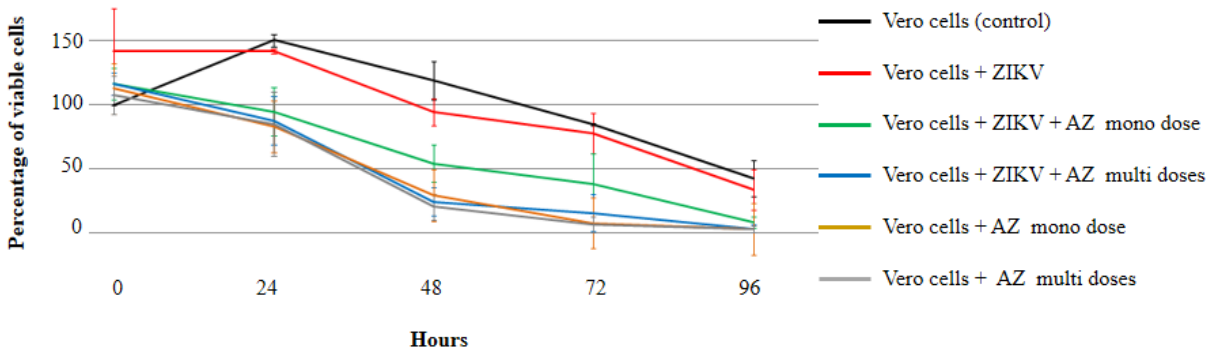

Figure 1: Cell viability assay. Percentage of viable cells at 0, 24, 48, 72 and 96 h for cells non-infected with ZIKV and non-treated with AZ (control); cells infected with ZIKV but non-treated with AZ; cells infected with ZIKV and treated with 50 mg/L of AZ in single dose or multi doses; and cells non-infected with ZIKV but treated with 50 mg/L of AZ in single dose or multi doses. Error bars are for standard deviation.

ZIKV RNA loads after treatment with single or multi doses of AZ at 50 mg/L

In the control experiment (cells infected with ZIKV but untreated with AZ), the mean ZIKV RNA load increased from 3.72 ± 0.12 to 6.40 ± 0.88 $\log_{10}$ copies/mL during 48 h post-infection, and then reached a plateau phase over 8 $\log_{10}$ copies/ml from 72 hpi (Table 2 and Figure 2).

Hours	Control (No AZ)	AZ single dose	AZ multi doses
0	3.72 ± 0.12	3.20 ± 0.25	3.08 ± 0.13
24	4.47 ± 0.12	3.49 ± 0.25	3.42 ± 0.17
48	6.40 ± 0.88	3.67 ± 0.16	3.50 ± 0.11
72	8.25 ± 0.11	4.97 ± 0.50	3.58 ± 0.17
96	8.63 ± 0.20	5.16 ± 0.37	3.71 ± 0.01

Table 2: Activity of AZ on ZIKV RNA replication in single dose and multi doses regimens. ZIKV RNA loads ($\log_{10}$ copies/mL, mean and standard deviation) were measured immediately and 24, 48, 72 and 96 h after infection of cells without treatment (control) and treatment with 50 mg/L of AZ in single dose or multi doses.

After addition of a single dose of AZ at 50 mg/L, mean ZIKV RNA loads were stable within 48 h post-treatment (range: 3.20 ± 0.25-3.67 ± 0.16 $\log_{10}$ copies/mL), and then increased to reach a plateau phase between 72 and 96 h post-treatment (range of 4.97 ± 0.50 to 5.16 ± 0.37 $\log_{10}$ copies/mL, respectively). ZIKV RNA loads at the plateau phase were more than 3 log lower compared to those detected for infected cells non-treated with AZ (control). With the multi doses protocol, ZIKV RNA loads were stable overtime between 0 and 96 h post treatment (range: 3.08 ± 0.13 to 3.71 ± 0.01 $\log_{10}$ copies/mL). These results suggest that a single addition of 50 mg/L of AZ delays RNA replication and sustained treatment is needed to prevent ZIKV RNA replication in a long-term period.

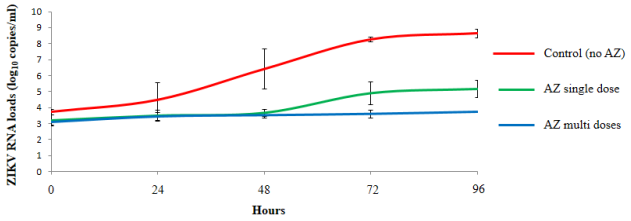

Figure 2: Activity of azithromycin on ZIKV in single dose and multi dose regimens. ZIKV RNA loads ($\log_{10}$ copies/mL) were measured immediately and 24, 48, 72 and 96 h after infection of cells and treatment with 50 mg/L of AZ in single dose or multi doses, or no treatment (control). Error bars are for standard deviation.

Detection of replicative ZIKV by IFA

For Vero cells infected with ZIKV but untreated with AZ (positive control), fluorescent signals were observed from 24 h post-infection (Figure 3). After addition of a single dose of AZ at 50 mg/L, fluorescence was detectable from 72 h post-treatment showing production of infectious ZIKV. No fluorescence was detected until 96 h post-treatment if using multi doses of AZ, confirming that multi doses of AZ at 50 mg/L abolished ZIKV replication in cells.

Discussion

Approved antiviral drugs are available to treat HIV, HCV, HBV, herpes simplex virus, varicella-zoster, *cytomegalovirus* and influenza viruses infections [18]. However there are no approved drugs for *Flavivirus* infections, even for DENV which is the most widespread arbovirus infection. Same as for the emergence of chikungunya virus (CHIKV) in the past decade, there is no therapeutic option to face the emergence of ZIKV.

The emergence of ZIKV in French Polynesia and subsequently in the Pacific and the Americas was associated with the description of severe neurological complications in adults (especially Guillain-Barré syndrome) and to a dramatic increase in fetuses/neonates central nervous system malformations (especially microcephaly) [1,2,19] To date, the only identified at-risk population for ZIKV infection is pregnant women because of potential complications for fetuses/neonates.

Mitigation strategies to prevent severe complication of ZIKV infections should focus on pregnant women: protection against mosquito bite, prevention of sexual transmission and postponing pregnancies in endemic areas; discouraging travel to endemic areas for pregnant women or women trying to conceive.

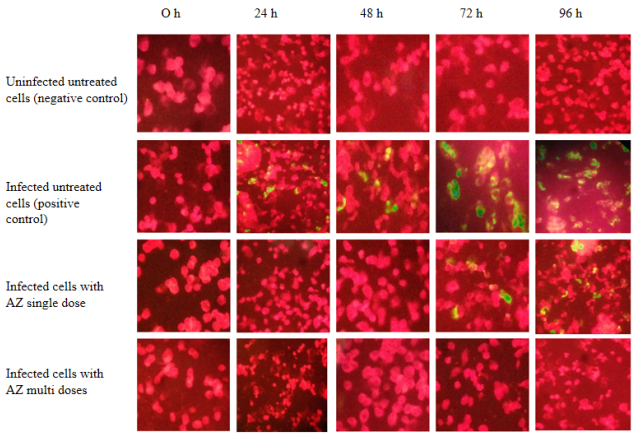

Figure 3: ZIKV detection in Vero cells by immunofluorescence assay at 24, 48, 72 and 96 h post infection and treatment with single dose or multi dose of AZ at 50 mg/L Infected cells are colored in fluorescent green whereas uninfected cells are colored in red.

Even if there are 45 vaccine projects under way, including nine ZIKV vaccine candidates that have entered phase I clinical trials and two that are in clinical trial phase II, we are far from the availability of a commercial vaccine, especially in the context of vaccination of pregnant women [4,5,20-22]. Anti ZIKV drugs inhibit ZIKV entry into cells [23-25]; inhibit viral replication targeting NS3 protein [26], NS5 protein [27], NS2B-NS3 protease [28], or nucleoside biosynthesis [29].

As there is an urgent need to face the emergence of ZIKV and to prevent/treat severe complications reported in adults and newborn/neonates, several strategies have been implemented to identify compounds with potential activity against ZIKV. A first strategy consists in drug repurposing by screening large libraries of compounds, including approved compounds [6-9,30]. This strategy detected active drugs on ZIKV but most of them cannot be used in pregnant women. A second strategy consists in restricting the research to approved drugs that can safely be used during pregnancy [7]. A third strategy consists in testing approved drugs with activity on other *flaviviruses* [31].

There is a broad range of drugs showing *in vitro* activity against ZIKV: anticancer and immunosuppressant, antiparasitic, inhibitor of activated caspases, antirheumatic, antibiotics (Daptomycine [6], AZ [7], and Kitasamycin [12], statin, iron chelators, Na+K+ ATPase inhibitor, sphingosine-1-phophate receptor modulator, antiparasitic, antihemetic, and antidepressant [3,8-10].

Recent studies demonstrated that antibiotics and antiparasitic agents could inhibit viral replication, especially *Flavivirus* and *Alphavirus* arboviruses [11]). Aglycon, an analog of teicoplanin was shown to be active on DENV, yellow fever, tick-borne encephalitis, Japanese encephalitis (JEV) and murine flavivirus Modoc viruses; ivermectin on CHIKV, yellow fever , dengue, Japanese encephalitis, tick-borne encephalitis, West Nile , CHIKV, Semliki Forest (SFV), and Sindbis viruses; niclosamide on JEV; abamectin on CHIKV; and AZ on ZIKV [6,7,9,11,32,33].

However there are some limitations for the use of these compounds to treat ZIKV infection in pregnant women: contraindication during pregnancy (i.e. azathioprine, cyclosporine A) or safety during pregnancy not demonstrated (i.e. clofazimine, Fingolimod). Other drugs, such as ivermectin or mefloquine, are only active at concentrations higher than the maximum concentration of the drug observed after its administration (Cmax) [6].

In our study we investigated the *in vitro* efficacy against ZIKV of AZ (new semisynthetic 15-membered ring azalide antibiotic belonging to the subclass of macrolides) [34] in a cell culture model because preliminary data suggested the efficacy of a singledose of AZ reduced the proliferation of ZIKV in glial cells [7]. AZ is a safe molecule during pregnancy [13,35,36]. AZ was found active *in vitro* to decrease the replication of *Rhinovirus* [37]. Animal studies and pregnant women follow-up demonstrated no evidence of damage for fetuses after gestational use of AZ. AZ is used as a single dose regimen for the treatment of sexually transmitted infections in pregnant women but damages were not documented after multi doses of AZ for the treatment of recurrent infections during pregnancy [35].

After the administration of a single dose at 1 g of AZ to pregnant women who underwent cesarean delivery, AZ concentrations 24 h post-medication were 27.1 ± 12.8 mg/L in umbilical cord serum, 36 ± 27 mg/L in amniotic fluid and 936 ± 223 mg/L in placenta [14]. In fetal specimens, the half-life of AZ was about 12 h in umbilical cord serum, 30 h in amniotic fluid, and 70 h in placenta [14]. Another study conducted with patients that underwent brain tumor removal showed that after a single dose of 500 mg of AZ, the ratio of concentration of the antibiotic in human brain tissues 24 h post-medication compared to blood was 255.76 ± 429 [38].

The main mechanism of action of AZ on bacteria is through binding with the 50s ribosomal subunit and inhibition of messenger RNA-directed polypeptide synthesis [34]. Hypotheses on the mechanism of action of macrolides on viruses are scarce. It was hypothesized that macrolides may inhibit respiratory syncytial virus through the reduced expression of fusion protein receptor, activated isoform A of the Ras-homologus (Rho) family, and the inhibition of subsequent Rho kinase activation in human airway epithelial cells [39].

Retallack et al. previously found that AZ reduced ZIKV proliferation in glial cells [7]. In their study, glial cells were pretreated for 1 h with AZ before infection with a clinical ZIKV strain from Puerto Rico. In our study we conducted additional experiments: we infected Vero cells with another ZIKV strain belonging to the Asian lineage, and Vero cells were infected before addition of AZ in order to determine the stage of the viral entry pathway in cells at which AZ can inhibit ZIKV replication. Moreover, we tested a multidose protocol during 4 days in addition to a single dose protocol.

Time of cell infection can suggest the mechanism of action of the drugs. Teicoplanin was shown to be active against Ebola virus by blocking virus entry because no inhibition was detected when the antibiotic was added after virus adsorption to the cell surface [40]; similar results were found with abamectin and ivermectin against CHIKV and SFV [41]. However, in the same assay, berberine (a plant-derived isoquinolone alkaloid) was active against CHIKV and SFV when added before and after cell infection [41].

Retallack et al. showed that AZ was active on ZIKV when added before cell infection [7]. Our study demonstrates that AZ is also active when added after ZIKV infection of Vero cells. Our data combined with those of Retallack et al. suggest that AZ is active *in vitro* when added before and after infection and can be potentially active *in vivo* to prevent and treat ZIKV infections. The strain used was isolated in French Polynesia, in which severe ZIKV infections complications were reported in fetuses/neonates and adults [1,42]. We demonstrated that the efficacy of AZ is not limited to the ZIKV Puerto Rico strain.

When comparing ZIKV replication in infected cells without AZ and in the single dose protocol we observed that AZ delayed the replication of ZIKV by 48 h and decreased the final amount of ZIKV RNA by more than 3 log. Macrolides are bacteriostatic agents that prevent the growth of bacteria while not killing them [34]. The inhibition of ZIKV replication after a single dose of AZ mimics a bacteriostatic curve. Although treatment with AZ decreased cell viability over time, the increased replication of ZIKV from 48 h after a single dose treatment suggests that the lack of ZIKV production was not due to the toxicity of AZ on cells. Daily addition of AZ inhibited the replication of ZIKV. This inhibition was superior in the AZ multidose protocol compared to the single dose regimen in our models.

Although our data confirm the activity of AZ on ZIKV replication, our study has some limitations. The mechanism of action of AZ on ZIKV is unknown [43]; we cannot speculate a mechanism by reference to the mechanism of action of macrolides or other antibiotics on ZIKV or other flaviviruses because all are unknown. In this study we used only one cell line model (Vero cells), while Retallack et al. used human glioblastoma astrocytoma cell line [7]; additional cell models, especially neuronal progenitor cells, should be tested. These data should also be confirmed in animal experimental models as *in vitro* antiviral activity is difficult to extrapolate to their possible use in humans [44].

Antiviral drugs showed absence of efficacy against recently emerging viruses [11,45]. Our work and previous studies open a new field in the fight against arboviruses, including ZIKV [46]. AZ is a safe molecule for pregnant women and may be of particular interest in the prevention of fetuses/neonates central nervous system complications of ZIKV infections. However, those *in vitro* data should be confirmed on animal models and further studies should be conducted to elucidate the mechanism of action of AZ on ZIKV.

References

1. Musso D, Gubler DJ (2016) Zika Virus. Clin Microbiol Rev 29: 487-524.
2. Baud D, Gubler DJ, Schaub B, Lanteri MC, Musso D (2017) An update on Zika virus infection. Lancet 390: 2099-2109.
3. Munjal A, Khandia R, Dhama K, Sachan S, Karthik K, et al. (2017) Advances in Developing Therapies to Combat Zika Virus: Current Knowledge and Future Perspectives. Front Microbiol 8: 1469.
4. Du L, Zhou Y, Jiang S (2017) The latest advancements in Zika virus vaccine development. Expert Rev Vaccines 16: 951-954.
5. Makhluf H, Shresta S (2018) Development of Zika Virus Vaccines. Vaccines 6. pii: E7.
6. Barrows NJ, Campos RK, Powell ST, Prasanth KR, Schott-Lerner G, et al. (2016) A Screen of FDA-Approved Drugs for Inhibitors of Zika Virus Infection. Cell Host Microbe 20: 259-270.
7. Retallack H, Di Lullo E, Arias C, Knopp KA, Laurie MT, et al. (2016) Zika virus cell tropism in the developing human brain and inhibition by azithromycin. Proc Natl Acad Sci 113: 14408-14413.
8. Pascoalino BS, Courtemanche G, Cordeiro MT, Gil LHVG, Freitas-Junior L (2016) Zika antiviral chemotherapy: identification of drugs and promising starting points for drug discovery from an FDA-approved library. F1000Res 5: 2523. eCollection.
9. Xu M, Lee EM, Wen Z, Cheng Y, Huang W-K, et al. (2016) Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. Nat Med 10: 1101-1107.
10. Balasubramanian A, Teramoto T, Kulkarni AA, Bhattacharjee AK, Padmanabhan R (2016) Antiviral activities of selected antimalarials against dengue virus type 2 and Zika virus. Antiviral Res 137: 141-150.
11. Colson P, Raoult D (2016) Fighting viruses with antibiotics: an overlooked path. Int J Antimicrob Agents 48: 349-352.
12. Saiz JC, Martín-Acebes MA (2017) The Race To Find Antivirals for Zika Virus. Antimicrob Agents Chemother 61. pii: e00411-17.
13. Lin KJ, Mitchell AA, Yau W-P, Louik C, Hernández-Díaz S (2013) Safety of macrolides during pregnancy. Am J Obstet Gynecol 208: 221.e1-8.
14. Ramsey PS, Vaules MB, Vasdev GM, Andrews WW, Ramin KD (2003) Maternal and transplacental pharmacokinetics of azithromycin. Am J Obstet Gynecol 188: 714-718.
15. Trösemeier JH, Musso D, Blümel J, Thézé J, Pybus OG, et al. (2016) Genome Sequence of a Candidate World Health Organization Reference Strain of Zika Virus for Nucleic Acid Testing. Genome Announc 4. pii: e00917-16.
16. Aubry M, Richard V, Green J, Broult J, Musso D (2016) Inactivation of Zika virus in plasma with amotosalen and ultraviolet A illumination. Transfusion 56: 33-40.
17. Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, et al. (2016) Cell Viability Assays. Assay guidance manual. Sittampalam GS, Coussens NP, Brimacombe K, et al. editors. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences. 2013, updated in 2016.
18. Menéndez-Arias L, Richman DD (2014) Editorial overview: antivirals and resistance: advances and challenges ahead. Curr Opin Virol 8: iv-vii.
19. Rubin EJ, Greene MF, Baden LR (2016) Zika Virus and Microcephaly. N Engl J Med 10: 984-985.
20. Dyer O (2016) Trials of Zika vaccine are set to begin in North America. BMJ 353: i3588.
21. Dowd KA, Ko S-Y, Morabito KM, Yang ES, Pelc RS, et al. (2016) Rapid development of a DNA vaccine for Zika virus. Science 354: 237-240.
22. Wahid B, Ali A, Rafique S, Idrees M (2017) Current status of therapeutic and vaccine approaches against Zika virus. Eur J Intern Med pii 44: 12-18.
23. Chen L, Liu Y, Wang S, Sun J, Wang P, et al. (2017) Antiviral activity of peptide inhibitors derived from the protein E stem against Japanese encephalitis and Zika viruses. Antiviral Res 141: 140-149.
24. Fernando S, Fernando T, Stefanik M, Eyer L, Ruzek D (2016) An Approach for Zika Virus Inhibition Using Homology Structure of the Envelope Protein. Mol Biotechnol 58: 801-816.

25. Delvecchio R, Higa L, Pezzuto P, Valadão A, Garcez P, et al. (2016) Chloroquine, an Endocytosis Blocking Agent, Inhibits Zika Virus Infection in Different Cell Models. Viruses 8 pii : E322.
26. Cao RY, Xu Y, Zhang TH, Yang JJ, Yuan Y, et al. (2017) Pediatric Drug Nitazoxanide: A Potential Choice for Control of Zika. Open Forum Infect Dis 4: ofx009.
27. Duan W, Song H, Wang H, Chai Y, Su C, et al. (2017) The crystal structure of Zika virus NS5 reveals conserved drug targets. EMBO 36: 919-933.
28. Lei J, Hansen G, Nitsche C, Klein CD, Zhang L, et al. (2016) Crystal structure of Zika virus NS2B-NS3 protease in complex with a boronate inhibitor. Science 353: 503-505.
29. Goebel S, Snyder B, Sellati T, Saeed M, Ptak R, et al. (2016) A sensitive virus yield assay for evaluation of Antivirals against Zika Virus. J Virol Methods 238: 13-20.
30. Devillers J (2018) Repurposing drugs for use against Zika virus infection. SAR QSAR Environ Res 29: 103-115.
31. Sahoo M, Jena L, Daf S, Kumar S (2016) Virtual Screening for Potential Inhibitors of NS3 Protein of Zika Virus. Genomics Inform 14: 104-111.
32. De Burghgraeve T, Kaptein SJF, Ayala-Nunez NV, Mondotte JA, Pastorino B, et al. (2012) An analogue of the antibiotic teicoplanin prevents flavivirus entry in vitro. PLoS One 7: e37244.
33. Mastrangelo E, Pezzullo M, De Burghgraeve T, Kaptein S, Pastorino B, et al. (2012) Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug. J Antimicrob Chemother 67: 1884-1894.
34. Jelić D, Antolović R (2016) From Erythromycin to Azithromycin and New Potential Ribosome-Binding Antimicrobials. Antibiotics (Basel) 5: pii: E29.
35. Sarkar M, Woodland C, Koren G, Einarson ARN (2006) Pregnancy outcome following gestational exposure to azithromycin. BMC Pregnancy Childbirth 6: 18.
36. Bérard A, Sheehy O, Zhao JP, Nordeng H (2015) Use of macrolides during pregnancy and the risk of birth defects: a population-based study. Pharmacoepidemiol Drug Saf 24: 1241-1248.
37. Gielen V, Johnston SL, Edwards MR (2010) Azithromycin induces anti-viral responses in bronchial epithelial cells. Eur Respir J 36: 646-654.
38. Jaruratanasirikul S, Hortiwakul R, Tantisarasart T, Phuenpathom N, Tussanasunthornwong S (1996) Distribution of azithromycin into brain tissue, cerebrospinal fluid, and aqueous humor of the eye. Antimicrob Agents Chemother 40: 825-826.
39. Asada M, Yoshida M, Suzuki T, Hatachi Y, Sasaki T, et al. (2009) Macrolide antibiotics inhibit respiratory syncytial virus infection in human airway epithelial cells. Antiviral Res 83: 191-200.
40. Wang Y, Cui R, Li G, Gao Q, Yuan S, et al. (2016) Teicoplanin inhibits Ebola pseudovirus infection in cell culture. Antiviral Re 125: 1-7.
41. Varghese FS, Kaukinen P, Gläsker S, Bespalov M, Hanski L, et al. (2016) Discovery of berberine, abamectin and ivermectin as antivirals against chikungunya and other alphaviruses. Antiviral Res 126: 117-124.
42. Besnard M, Eyrolle-Guignot D, Guillemette-Artur P, Lastère S, Bost-Bezeaud F, et al. (2016) Congenital cerebral malformations and dysfunction in fetuses and newborns following the 2013 to 2014 Zika virus epidemic in French Polynesia. Euro Surveill 21.
43. Iannetta M, Ippolito G, Nicastri E (2017) Azithromycin Shows Anti-Zika Virus Activity in Human Glial Cells. Antimicrob Agents Chemother 6: e01152-17.
44. Saiz J-C, Martín-Acebes MA (2017) Reply to Iannetta et al., "Azithromycin Shows Anti-Zika Virus Activity in Human Glial Cells". Antimicrob Agents Chemother 61: e01192-17.
45. Bekerman E, Einav S (2015) Infectious disease. Combating emerging viral threats. Science 348: 282-283.
46. Raoult D (2017) A double paradigm shift for the antibiotics' activity on viruses: Zika' lesson. Proc Natl Acad Sci U S A 114: E1045.