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Randomized, phase 1/2, double-blind pioglitazone repositioning trial combined with antifungals for the treatment of cryptococcal meningitis – PIO study

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ABSTRACT

Background: Cryptococcosis affects more than 220,000 patients/year, with high mortality even when the standard treatment [amphotericin B (AMB), 5-flucytosin (5-FC) and fluconazole] is used. AMB presents high toxicity and 5-FC is not currently available in Brazil. In a pre-clinical study, pioglitazone (PIO - an antidiabetic drug) decreased AMB toxicity and lead to an increased mice survival, reduced morbidity and fungal burden in brain and lungs. The aim of this trial is to evaluate the efficacy and safety of PIO combined with standard antifungal treatment for human cryptococcosis.

Methods: A phase 1/2, randomized, double blind, placebo-controlled trial will be performed with patients from Belo Horizonte, Brazil. They will be divided into three groups (placebo, PIO 15 mg/day or PIO 45 mg/day) and will receive an additional pill during the induction phase of cryptococcosis' treatment. Our hypothesis is that treated patients will have increased survival, so the primary outcome will be the mortality rate. Patients will be monitored for survival, side effects, fungal burden and inflammatory mediators in blood and cerebrospinal fluid. The follow up will occur for up 60 days.

Conclusions: We expect that PIO will be an adequate adjuvant to the standard cryptococcosis' treatment.

Trial registration: ICTRP/WHO (and International Clinical Trial Registry Plataform (ICTRP/WHO) (http://apps.who.int/trialsearch/Trial2.aspx?TrialID=RBR-9fv3f4), RBR-9fv3f4 (http://www.ensaiosclinicos.gov.br/rg/RBR-9fv3f4). UTN Number: U1111-1226-1535. Ethical approvement number: CAAE 17377019.0.0000.5149.

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1. Introduction

Cryptococcosis, a severe systemic mycosis that affects lungs and brain caused by *Cryptococcus neoformans* and *C. gattii*, accounts for 223,100 cases/year with 81% mortality [1,2]. It is considered as the fifth infectious disease in terms of lethality in the world, following AIDS, tuberculosis, malaria and diarrhea [3]. Neurocryptococcosis, the most severe form of the disease, has been associated with 15% of deaths in HIV patients [4], but the infection also occurs in immunocompetent individuals [5,6].

The gold standard anticryptococcal therapy is the combination of amphotericin B (AMB) and 5-flucytosin (5-FC) during induction phase, followed by consolidation and maintenance with fluconazole (FLC). Several problems are associated to this therapy: AMB toxicity; unavailability of 5-FC in many countries, such as Brazil; and FLC antifungal resistance [7]. The liposomal formulation of AMB is less toxic but is unaffordable for most of health systems [8]. Another recurrent problem during the treatment is the Immune Reconstitution Inflammatory Syndrome (IRIS), i.e. an exacerbated immune response, which contributes to the high mortality rate of cryptococcosis [9].

Considering the important role and toxicity of AMB, we searched for an adjuvant drug that could reduce its side effects and improve patients' physiology, aiming a better response to the treatment. We used the concept of drug repurposing, which is based on the use of an established drug (with known pharmacology and toxicology) for a new useful indication [10]. In this context, several studies demonstrated that the antidiabetic PPARγ agonist pioglitazone (PIO) has anti-inflammatory effects [11], and increases host tolerance in malaria by activation of pathways that leads to neuroprotective and neuroregenerative effects. These effects are also useful for central nervous system (CNS) injury, stroke and ischemia [12]. In addition, several clinical trials demonstrated the efficacy and safety of PIO as an adjuvant in cancer therapies, autism and also in cardiometabolic studies [13–23].

Based on that, we tested PIO combined with AMB in murine model of cryptococcosis. PIO reduced AMB toxicity by decreasing creatinine and liver transaminase levels, improving mice's physiology. Overall, PIO + AMB had additional benefits than AMB alone, with increased mice survival, decreased morbidity and reduced fungal burden, without altering blood glucose levels [11].

In this paper we describe the protocol that will be followed to evaluate the efficacy and safety of PIO (combined with standard antifungal treatment) in treating *Cryptococcus* infection in Brazilian patients in a double-blind, randomized, phase 1/2 study. Cryptococcosis patients will be treated daily with standard Brazilian therapy (AMB + FLC) plus PIO (15 or 45 mg/day) or a placebo formulation during the induction phase of the treatment. All patients will be evaluated regarding survival, fungal burden in plasma and cerebrospinal fluid (CSF), inflammatory response and clinical and laboratory monitoring. We expect reduced AMB side effects, decreased patient mortality and sequels.

2. Methods

2.1. Study design

This protocol, named as *PIO study*, has been designed as a double-blind, phase 1/2, randomized, placebo-controlled clinical trial for evaluating the safety and efficacy of PIO as an adjuvant to the treatment of cryptococcosis. The trial will be performed at Eduardo de Menezes Hospital (EMH), a referral hospital for infectious diseases, which belongs to the Foundation's assistance network of *Fundação Hospitalar do Estado de Minas Gerais* (FHEMIG), in Belo Horizonte, Minas Gerais State, Brazil. 57 cryptococcosis patients will be included; all of them will receive the hospital's standard therapy, having a placebo or PIO added during the induction phase (first 14 days) of treatment. Patients will be divided into three groups: 1) placebo; 2) PIO 15 mg/day and 3) PIO 45 mg/day. The test drug and placebo have been developed to be as similar as possible, having the same color and will be placed in identical flasks, which will be sequentially labeled by numbers. Each flask will have the exact number of tablets required for treatment throughout the study and will be delivered to the hospital staff, which will record the flask number in the patient's form. Fig. 1 shows a flow chart of the steps to be performed during the clinical trial. The data will be analyzed regarding the patient's survival, fungal burden in plasma and CSF, inflammatory response, and clinical and laboratorial monitoring of adverse effects.

At days 0 (before treatment), 3, 10 and 14 days after the beginning of treatment, blood will be collected for C-reactive protein assay, liver function tests (such as bilirubin, alkaline phosphatase, aminotransferases, albumin and prothrombin time), renal function tests (such as creatinine and urea), and-HCG (for women). Blood and CSF will also be used to determine fungal burden by counting the number of colony forming units (CFU/mL). This procedure will allow comparing the efficiency of placebo and control treatments in reducing AMB toxicity and in eliminating the fungus. For fungal burden determination, 100 μL of CSF and blood will be serial diluted and plated on Sabouraud Dextrose Agar (SDA). Colonies will be counted after 48 h of incubation at 37 °C to quantify CFU/mL. All the data obtained (CFU and all laboratorial results) will be evaluated i) for the same patient to monitor fungal load and drug toxicity during treatment and ii) also to compare patients from different study groups. *Cryptococcus* clearance in CSF and blood occurs when no fungal colonies are detected during the assay [24]. We also aim to evaluate the inflammatory response in CSF and blood by measuring the levels of the mediators IL-10, IL-6, IFN-γ, TNF-α and CXCL1 by ELISA.

2.2. Sample size

According to previous data [25], the mortality rate of patients 10 weeks after the beginning of treatment is 41.2%. In our pre-clinical study for PIO repositioning, the survival after 10 wees of the protocol was about 100% [11]. The current mortality rate of cryptococcosis patients at the EMH is 29.8%, which corresponds to a success rate of 70.2%. Based on this data, the success rate of 70.2% was chosen for calculating the sample size [26].

The following parameters were used for the calculation: confidence level of 95%; Power of 80%; success rate of Cryptococcosis treatment at EMH of 70%; success rate of cryptococcosis treatment in our pre-clinical study for PIO repositioning at 10 weeks of 100%.

Considering the parameters mentioned above, the following formula (https://select-statistics.co.uk/calculators/sample-size-calculator-two-proportions/) was used:

$$N = \left(Z_{\alpha/2} + Z_{\beta}\right)^2 * \left(p_1(1-p_1) + p_2(1-p_2)\right) / \left(p_1 - p_2\right)^2$$

Where:

N: target sample number
Zα/2: 1.96 (the critical value of the Normal distribution at α/2, considering 5% error and the confidence interval of 95%)
Zβ: 0.84 (the critical value of the Normal distribution at β, considering a power of 80%)
p1: 0.702 (success fraction observed for EMH patients)
p2: 1(success fraction observed in the pre-clinical study)

Applying these values to the formula, the sample size calculated is at least 19 patients per group.

2.3. Recruitment

Patients aged ≥18 years and diagnosed with cryptococcosis will be enrolled to be included in the study. Patients can be either HIV-infected or HIV-negative. Patients (or responsible) will sign the informed consent form to be included in the study.

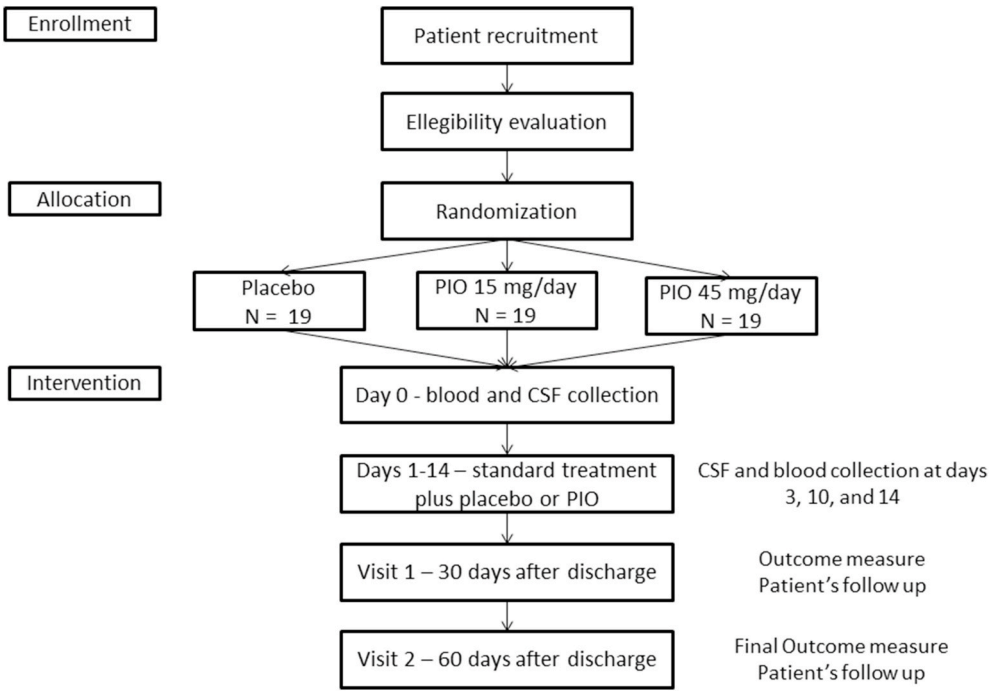

Fig. 1. Study flowchart. Fifty seven patients with neurocryptococcosis admitted to Eduardo de Menezes Hospital, and those who meet the criteria will be enrolled in the study. All of them will receive the hospital standard therapy, having a placebo or PIO added during the induction phase of treatment. Patients will be divided into three groups: 1) placebo; 2) PIO 15 mg/day and 3) PIO 45 mg/day. Data will be analyzed regarding patients' survival, fungal burden in plasma and CSF collected during treatment with PIO, clinical and laboratorial monitoring of adverse effects and patients' follow up after discharge.

2.3.1. Exclusion criteria

The exclusion criteria are: hypersensitivity to PIO; age bellow 18 years; diabetes mellitus; pregnancy; renal failure; patients on dialysis; patients with active liver failure or increased glutamic-pyruvic transaminase - GTP (higher than five times the normal range); history of bladder tumors; relapsed patients with previous antifungal treatment; patients using drugs that may interfere with outcomes such as antifungals or drugs already repositioned as adjuvants to antifungal therapy (e.g. sertraline, amiodarone and atorvastatin); unconscious patients (unable to swallow the pills); hypoglycemia; congestive heart failure of any level and patients with segmental deficit, ejection fraction less than 40%, atrial fibrillation and dilated cardiomyopathy.

2.4. Randomization and allocation

Random assignment will be performed by using a computer-generated list of a blocked randomization sequence, with block sizes of 3 and 6 patients. Participants will be allocated to each group at a 1:1:1 ratio. The record of the numbers will be kept in the Mycology Lab, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

2.5. Interventions

All eligible patients will receive the hospital standard therapy plus one tablet according to the allocated group, placebo, PIO 15 mg/day or PIO 45 mg/day during the induction phase (first 14 days of treatment). The standard treatment differs depending on the species isolated (*C. neoformans* or *C. gattii*) and whether the patient is HIV-positive or negative. The drugs currently available in EMH are AMB and FLC. AMB is available in formulations with deoxycholate, lipid and liposomal complex. However, lipid and liposomal formulations are only used in cases of renal failure (creatinine clearance $\leq$50 mL/min), which is an exclusion criterion. The treatment is divided into the induction, consolidation and maintenance phases. The induction phase aims efficient pathogen elimination and, in EMH, is performed with the combination of AMB and FLC. Negative CSF culture is expected at the end of induction therapy, and the patient may be discharged if conditions other than cryptococcosis do not exist. The maintenance and consolidation phases are performed with FLC. Doses and duration are as follows, for HIV-positive patients infected with *C. neoformans*, which corresponds to the most of cases:

Induction phase: AMB deoxycholate 0.7–1 mg/kg (maximum 50 mg/day) + Fluconazole 800 mg/day orally or intravenously for at least 14 days. Treatment may last 14 days as long as *Cryptococcus* CSF culture harvested at or after the 14th day of treatment reveals a negative result.

Continuation of this induction therapy beyond two weeks may be considered for additional weeks (1–6) if:

(1) presence of cerebral cryptococcoma evidenced by magnetic resonance imaging (MRI) (in this case the induction phase will last 6 weeks);
(2) the patient is clinically worsening;
(3) the patient has not improved and maintains persistently elevated and symptomatic intracranial pressure;
(4) CSF culture for *Cryptococcus* obtained after two weeks of induction therapy remains positive;
(5) the patient is in a coma.

If CSF culture after two weeks of treatment is reported as positive after discontinuation of the induction regimen, reintroduction of at least another two-week induction course may be considered. In this case, PIO or placebo administration will be suspended after 14 days and patient will follow hospital's protocol.

In the consolidation phase, patients take FLC 800 mg/day orally for eight weeks. In the maintenance phase, patients use fluconazole 200 mg–400 mg orally for 12 months and discontinue only after undetectable viral (in HIV patients) load and two CD4 $>$ 100 results at three-months interval.

Patients with different clinical aspects (*C. gattii* infections and/or HIV negative) will be included in the study, but the standard therapy differs according to each clinical case, as stated in the hospital standard protocol.

2.6. Outcome measurement

2.6.1. Primary outcomes

Our hypothesis is that treated patients will have increased survival, so the primary outcome will be the mortality rate.

2.6.2. Secondary outcomes

The secondary outcomes expected are:

- Reduction of CSF fungal burden at the beginning of treatment;
- Negative CSF culture after 14 days;
- Lower occurrence of IRIS;
- Reduction of relapse;
- Reduction of serious adverse effects;
- Reduction in levels of inflammatory mediators in blood and CSF.

The cure criterion to be used for discharge is the negativity of CSF culture (if no other comorbidity exists). After being discharged, patients will return to the hospital once a month for two months (around 10 weeks) to certify adherence to the maintenance treatment.

2.6.3. Safety outcomes

Participants will be monitored daily, and at days 0, 3, 10 and 14 after the beginning of treatment, CSF and blood will be collected. As described above, liver, heart and kidney functions will be assessed. Furthermore, all adverse effects will be reported throughout the study period and will be documented on the patient's form.

Side effects from PIO usually occur in less than 5% of patients on continuous drug use [25]. Effects are less likely to occur when the drug is used for short periods. The main side effects are edema, increased body weight, reduced hemoglobin and hematocrit levels, increased creatine kinase, macular edema, and bone fractures in women. All patients, while using PIO or placebo, will be admitted to the EMH and will have all assistance from the care staff, so that electrocardiogram and laboratory tests and therapeutic interventions will be performed whenever necessary.

The occurrence of edema will be assessed by clinical observation through palpation. Laboratory tests will be performed to evaluate hemoglobin and hematocrit levels, in addition to creatine kinase activity. All results of laboratory tests performed during treatment will be compared with those performed at the time of admission of the patient. Hematocrit values lower than 35% for women and than 40% for men, as well as hemoglobin levels below 11 g/dL will be considered as a signal for verifying discontinuation of PIO, if these levels were not detected at the admission time. Creatine kinase values greater than 200 U/L will also be considered as an alert for the need to discontinue the use of PIO.

In all cases where severe adverse events (cardiac, hepatic or renal) or hypersensitivity to PIO is observed, the patient will be discontinued from the study. In this case, the patient will be monitored clinically, and appropriate measures will be taken to reverse the detected side effect. Adverse effects will be ranked according to the National Institute of Allergy and Infectious Diseases (NIAID) AIDS toxicity scale, 2017 version, to assess adverse events in all participants [28]. Due to an expected incidence of 80% of Grade 3-4 adverse events with deoxycholated AMB, Grade 4–5 adverse events will be considered [29].

2.7. Study termination and withdrawal criteria

Withdrawal and study termination criteria are the patient or legal representative withdrawals consent to participate in the study; serious adverse effects (whether cardiac, hepatic or renal, as evidenced by clinical and laboratory examinations and reported in the prior state) and hypersensitivity to PIO.

2.8. Statistical analysis

Statistical analysis will be performed by using GraphPad Prism, version 6.00, for Windows (GraphPad Software, San Diego, CA, USA) with $P < 0.05$ considered statistically significant. Assessment of patients' survival will be performed by constructing the Kaplan-Meier curve and the treated and placebo groups will be compared with each other. The groups will be compared by using the log-rank test, and Gehan–Breslow–Wilcoxon test. The parameters "CSF fungal burden" and "inflammatory mediators" will be compared for the same patient at different times, and also between patients from different groups (means $\pm$ SD) considering the same analysis time. The test to be used will be the analysis of variance (Kruskal-Wallis) with Dunn's posttest. In addition to the comparison between groups, longitudinal analysis will also be performed (to assess CSF clearance and levels of inflammatory mediators), through the construction of dispersion curves and regression and correlation analysis (the test to be applied will be defined according to the profile of each curve, which may be linear, polynomial or logarithmic). Specifically for the analysis of inflammatory mediators, the level of proinflammatory (IL-6, IFN-γ, TNF-α, and CXCL1) and anti-inflammatory (IL-10) mediators in each patient at different time points will be compared; this analysis will also be performed between the treatment groups and the placebo group. Clinical progression of patients and the occurrence of adverse events during therapy will be documented in the medical records qualitatively (presence or absence) and the data will be analyzed using the chi-square test. Patients' follow-up laboratory test results will be evaluated by analysis of variance (Kruskal-Wallis) with Dunn's posttest. The Bonferroni test will be used for controlling multiplicity.

2.9. Quality control

The clinical trial will be monitored by the Mycology Lab group. Confidentiality and all personal information belonging to the patients will be protected during and after the clinical trial. The researchers responsible for the study, as well as the entire team involved, are committed to treating the patient's identity under professional standards of confidentiality, privacy and image protection. The identity of patients who agree to participate in the research will be kept confidential and will not be disclosed in any publication that may be generated in this study, as well as possible subsequent studies. The entire project will be developed in accordance with the rules and laws governing the use of human material, in accordance with the criteria of the Resolution No. 466 (December 12, 2012) of the National Health Council and its complementary rules and resolutions. For ensuring the quality of the study, both placebo and PIO will be manufactured on a single batch, and quality control of the drugs will be independently performed using standard methods.

2.10. Ethics approval and dissemination

The project has been approved by the Brazilian National Research Ethics Committee (Comissao Nacional de Etica em Pesquisa - CONEP) (CAAE number: 17377019.0.0000.5149) and registered at the Brazilian Clinical Trials Registry/Registro Brasileiro de Ensaios Clínicos (ReBEC) (http://www.ensaiosclinicos.gov.br/rg/RBR-9fv3f4/) and International Clinical Trial Registry Plataform (ICTRP/WHO) (http://apps.who.int/trialsearch/Trial2.aspx?TrialID=RBR-9fv3f4).

3. Discussion

PIO has been shown to be a promising adjuvant for the treatment of cryptococcosis in murine model, since its association with AMB reduced toxicity, allowing an increased mice survival and decreased fungal burden in lungs and brain [11]. Despite the promising results, no clinical trial has been performed so far in cryptococcosis patients. Considering

that (i) AMB is the first-choice drug for induction phase in patients in Brazil and the (ii) high toxicity of this drug, our aim is to use PIO as an antidote for the AMB nephrotoxicity. We believe that a lower toxicity will improve the patients' physiology, allowing faster fungal killing and reduced mortality rate. This is the first clinical trial with PIO for treatment of cryptococcosis in human patients and may provide an alternative for increasing the survival rate of patients who suffer from this disease. The results are expected to contribute to the establishment of less toxic therapy for cryptococcosis.

Author contributions

Conceptualization: DAS, LGE, VCRM, LSP, GPA.
Data curation: DAS, LGE, VCRM, LSP, GPA, NTAP.
Funding acquisition: DAS.
Investigation: DAS, LGE, NQR, JRAS, MCC, ECPE, GJCF, PHFC, BAM, JCMDO, LMVS, VATL, VCRM, LSP, IP, LFR, FAAF, MRTD, MA, LP, DIS, MGTM, VAAZ, AAGF, ICC, GPA, LFCM, NTAP.
Methodology: DAS, LGE, NQR, JRAS, MCC, ECPE, GJCF, PHFC, BAM, JCMDO, LMVS, VATL, VCRM, LSP, IP, LFR, FAAF, MRTD, MA, LP, DIS, MGTM, VAAZ, AAGF, ICC, GPA, LFCM, NTAP.
Project administration: DAS, NTAP, VCRM, LSP, LFCM, GPA.
Supervision: DAS, NTAP, VCRM, LSP, LFCM, GPA.
Writing – original draft: LGE, NQR, DAS.
Writing – review and editing: LGE, NQR, DAS.

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References

[1] R. Rajasingham, R.M. Smith, B.J. Park, et al., Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis, Lancet Infect. Dis. 17 (8) (2017) 873–881.
[2] P. Williamson, J. Jarvis, A. Panackal, et al., Cryptococcal meningitis: epidemiology, immunology, diagnosis and therapy, Nat. Rev. Neurol. 13 (1) (2017) 13–24.
[3] M.L. Rodrigues, P.C. Albuquerque, Searching for a change: the need for increased support for public health and research on fungal diseases, PLoS Neglected Trop. Dis. 12 (6) (2018) 1–5.
[4] R. Rajasingham, R.M. Smith, B.J. Park, et al., Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis, Lancet Infect. Dis. 17 (2017) 873–881.
[5] V. Chaturvedi, S. Chaturvedi, *Cryptococcus gattii*: a resurgent fungal pathogen, submitted for publication, Trends Microbiol. 19 (2011) 564–571.
[6] J.C. Hurtado, P. Castillo, F. Fernandes, et al., Mortality due to *Cryptococcus neoformans* and *Cryptococcus gattii* in low-income settings: an autopsy study, Sci. Rep. 9 (2019) 1–10.
[7] J.R. Santos, N.Q. Ribeiro, R.W. Bastos, et al., High-dose fluconazole in combination with amphotericin B is more efficient than monotherapy in murine model of cryptococcosis, Sci. Rep. 7 (2017) 1–10.
[8] J. Santos-Gandelman, M.L. Rodrigues, A. Machado, Future perspectives for cryptococcosis treatment, Expert Opin. Ther. Pat. 28 (2018) 625–634.
[9] S.C.A. Chen, T.M. Korman, M.A. Slavin, et al., Antifungal therapy and management of complications of cryptococcosis due to *Cryptococcus gattii*, Clin. Infect. Dis. 22 (2013) 1–33.
[10] N.Q. Ribeiro, M.C. Costa, T.F.F. Magalhaes, et al., Atorvastatin as A promising anticryptococcal agent, Int. J. Antimicrob. Agents 49 (2017) 695–702.
[11] N.Q. Ribeiro, A.P.N. Santos, E.C.P. Emídio, et al., Pioglitazone as an adjuvant of Amphotericin B for the treatment of cryptococcosis, Int. J. Antimicrob. Agents 54 (2019) 301–308.
[12] L. Serghides, C.R. McDonald, Z. Lu, et al., PPARγ agonists improve survival and neurocognitive outcomes in experimental cerebral malaria and induce neuroprotective pathways in human malária, PLoS Pathog. 10 (2014) 1–14.
[13] P. Hau, L. Kunz-Schughart, U. Bogdahn, et al., Low-dose chemotherapy in combination with COX-2 inhibitors and PPAR-gamma agonists in recurrent high-grade gliomas - a phase II study, Oncology 73 (2007) 21–25.
[14] K.G. Tolman, J.W. Freston, S. Kupfer, et al., Liver safety in patients with type 2 diabetes treated with pioglitazone: results from a 3-year, randomized, comparator-controlled study in the US. Randomized controlled trial, Drug Saf. 32 (2009) 787–800.
[15] R.E. Ryder, Pioglitazone: reports of its death are greatly exaggerated - it is alive and ready to resume saving lives, Diabet. Med. 32 (2015) e9–e15.
[16] L. Capano, A. Dupuis, J. Brian, et al., A pilot dose finding study of pioglitazone in autistic children, Mol. Autism. 9 (2018) 1–14.
[17] L.H. Young, C.M. Viscoli, G.G. Schwartz, et al., Heart failure after ischemic stroke or transient ischemic attack in insulin-resistant patients without diabetes mellitus treated with pioglitazone, Circulation 138 (2018) 1210–1220.
[18] V. Pavlova, E. Filipova, K. Uzunova, et al., Pioglitazone therapy and fractures: systematic review and meta- analysis, Endocr. Metab. Immune Disord. - Drug Targets 18 (2018) 502–507.
[19] A. Tanaka, S. Komukai, Y. Shibata, et al., Effect of pioglitazone on cardiometabolic profiles and safety in patients with type 2 diabetes undergoing percutaneous coronary artery intervention: a prospective, multicenter, randomized trial, Heart Ves. 33 (2018) 965–977.
[20] C.K. Cramer, N. Alphonse-Sullivan, S. Isom, et al., Safety of pioglitazone during and after radiation therapy in patients with brain tumors: a phase I clinical trial, J. Canc. Res. Clin. Oncol. 145 (2019) 337–344.
[21] M. Ghadiany, M. Tabarraee, S. Salari, et al., Adding oral pioglitazone to standard induction chemotherapy of acute myeloid leukemia: a randomized clinical trial, Clin. Lymphoma, Myeloma & Leukemia 19 (2019) 206–212.
[22] P. Pourshabanan, A. Momeni, L. Mahmoudnia, S. Kheiri, Effect of pioglitazone on decreasing of proteinuria in type 2 diabetic patients with nephropathy, Diabetes Metab Syndr 13 (2019) 132–136.
[23] J.D. Spence, C.M. Viscoli, S.E. Inzucchi, et al., Pioglitazone therapy in patients with stroke and prediabetes: a post hoc analysis of the IRIS randomized clinical trial, JAMA Neurol 76 (2019) 526–535, https://doi.org/10.1001/jamaneurol.2019.0079.
[24] J. Dyal, A. Akampurira, J. Rhein, et al., Reproducibility of CSF quantitative culture methods for estimating rate of clearance in cryptococcal meningitis, Med. Mycol. 54 (2016) 361–369.
[25] M.W. Tenforde, A.E. Shapiro, B. Rouse, et al., Treatment for HIV-associated cryptococcal meningitis, Cochrane 7 (2018) 159.
[26] G.R.S. Weyne, Determinação do tamanho da amostra em pesquisas experimentais na área de saúde, Arq. Med. ABC 29 (2004) 87–90.
[28] U.S. Department Of Health And Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS, Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017.
[29] J. Rhein, B.M. Morawski, K.H. Hullsiek, et al., Efficacy of adjunctive sertraline for the treatment of HIV-associated cryptococcal meningitis: an open-label dose-ranging study, Lancet Infect. Dis. 16 (2016) 809–818.