Nonclinical evaluation of IQG-607, an anti-tuberculosis candidate with potential use in combination drug therapy

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ABSTRACT

New effective compounds to treat tuberculosis are urgently needed. IQG-607 is an orally active anti-tuberculosis drug candidate, with promising preliminary safety profile and anti-mycobacterial activity in both *in vitro* and *in vivo* models of tuberculosis infection. Here, we evaluated the mutagenic and genotoxic effects of IQG-607, and its interactions with CYP450 isoforms. Moreover, we describe for the first time a combination study of IQG-607 in *Mycobacterium tuberculosis*-infected mice. Importantly, IQG-607 had additive effects when combined with the first-line anti-tuberculosis drugs rifampin and pyrazinamide in mice. IQG-607 presented weak to moderate inhibitory potential against CYP450 isoforms 3A4, 1A2, 2C9, 2C19, 2D6, and 2E1. The *Salmonella* mutagenicity test revealed that IQG-607 induced base pair substitution mutations in the strains TA100 and TA1535. However, in the presence of human metabolic S9 fraction, no mutagenic effect was detected in any strain. Additionally, IQG-607 did not increase micronucleus frequencies in mice, at any dose tested, 25, 100, or 250 mg/kg. The favorable activity in combination with first-line drugs and mild to moderate toxic events described in this study suggest that IQG-607 represents a candidate for clinical development.

1. Introduction

Tuberculosis is the leading cause of death from a single infectious agent, *Mycobacterium tuberculosis*, and killed approximately 1.6 million people in 2017 (WHO, 2018). Despite some progress in the pipeline for new drug candidates and regimens, there still is a need for developing new chemical compounds and strategies to treat tuberculosis (Pai et al., 2016; WHO, 2018). Ideally, a new anti-tuberculosis drug should be orally administered and included in a shorter and less toxic regimen to improve patients' compliance (Pai et al., 2016).

IQG-607, a molecule consisting of a pentacyanoferrate moiety attached to the nitrogen atom of the heterocyclic ring of isoniazid, is an inhibitor of the 2-*trans*-enoyl-ACP reductase enzyme (Oliveira et al., 2004, 2006; Vasconcelos et al., 2008; Abbadi et al., 2018), which blocks the biosynthesis of mycolic acids in *M. tuberculosis* (Rodrigues-Junior et al., 2014). Importantly, we have reported promising data showing the biological activity of IQG-607 in models of tuberculosis infection both *in vitro* (Basso et al., 2010; Rodrigues-Junior et al., 2014) and *in vivo* (Rodrigues-Junior et al., 2012). IQG-607 given by the oral route to *M. tuberculosis*-infected mice reduced lung lesions and diminished bacterial burden in spleens and lungs (Rodrigues-Junior et al., 2012). Additionally, experiments using early and late controls of infection demonstrated a bactericidal effect of IQG-607 (Rodrigues-Junior et al., 2012).

IQG-607 was not cytotoxic for eukaryotic cell lineages and did not exhibit *in vitro* genotoxic effects in HepG2 cells as shown by the alkaline comet assay (Amorim et al., 2017). Animal experiments revealed low toxicity when IQG-607 was acutely administered to mice (Basso et al., 2010). Moreover, we found only *mild to moderate toxic events* in Wistar rats (Rodrigues-Junior et al., 2017a) and in minipigs (Rodrigues-Junior

et al., 2017b), after acute and repeated schedules of IQG-607 administration. *IQG-607 satisfactory in vivo activity and* favorable toxicological profiles *in both rodent and non-rodents encouraged us to perform further nonclinical studies with this promising drug candidate.* In this work, we evaluated the possible mutagenic and genotoxic effects of IQG-607, as well as its possible interactions with CYP450 isoforms. We also describe a combination therapy of IQG-607 with clinically used antitubercular drugs in a mouse model of tuberculosis.

2. Materials and methods

2.1. Drug combination in Mycobacterium tuberculosis-infected mice

2.1.1. Drugs and bacteria

IQG-607 was synthesized according to Oliveira et al. (2006). Rifampin and pyrazinamide were purchased from Sigma-Aldrich, and isoniazid (INH) was purchased from ACROS Organics. Rifampin and pyrazinamide stock solutions were prepared weekly in distilled water and stored at 4 °C, as described in previous protocols (Almeida et al., 2009; Grosset et al., 2012). INH and IQG-607 solutions were freshly prepared in distilled water prior to administrations. *M. tuberculosis* H37Rv strain was cultured as previously described (Rodrigues-Junior et al., 2012).

2.1.2. Mice

For infections, male CF1 mice (24–30 g) obtained from the Central Biotery of PUCRS (CeMBE, Brazil) were employed. This experiment was performed at Preclinical Tests Laboratory, located at CPBMF-PUCRS. Animals were maintained at temperature-(22 ± 1 °C) and humidity-controlled room, with a 12/12 h light/dark cycle, and food and water available *ad libitum*. The current Brazilian guidelines for the care and use of animals for scientific and academic student training procedures, from the National Council for the Control of Animal Experimentation (CONCEA, Brazil), was followed. This experimental protocol was previously approved by the Animal Ethics Committee of PUCRS (CEUA protocol numbers 16/7348 and 18/8637).

2.1.3. Mice infection and treatments

Thirty CF1 mice were anaesthetized and intravenously infected with 10^6 *M. tuberculosis* H37Rv, according to the technique previously described (Grosset et al., 1992; Rodrigues-Junior et al., 2012). Fourteen days post-infection, mice were randomly divided into five groups with six animals each and treatments started (Grosset et al., 1992). A group of animals, named early control group, was sacrificed at the day of treatment initiation to determine the pre-treatment colony-forming units (CFU) counts. Three experimental groups received the following treatments with different drug combinations: rifampin, 10 mg/kg plus pyrazinamide, 150 mg/kg (RZ); rifampin, 10 mg/kg plus pyrazinamide, 150 mg/kg plus isoniazid, 25 mg/kg (RZ + INH); or rifampin, 10 mg/kg plus pyrazinamide, 150 mg/kg plus IQG-607, 25 mg/kg (RZ + IQG-607). The doses of rifampin, pyrazinamide and INH were chosen to achieve serum antibiotic concentrations in mice equivalent to those obtained with current recommended antibiotic therapy for humans (Grosset et al., 1992, 2012; Almeida et al., 2009). Drugs were administered by oral gavage five times weekly for 4 weeks. Rifampin was given 1 h before administration of other drugs to avoid any adverse pharmacokinetic interaction (Almeida et al., 2009; Grosset et al., 2012). An untreated control group received the vehicle (distilled water) during the treatment period.

2.1.4. Assessment of treatment efficacy

Treatment efficacy was assessed by determining lung and spleen CFU counts. Mice were euthanized by sevoflurane (Cristália) inhalation, three days after the last administrations, and spleens and left lungs were removed under aseptic conditions. Both organs were placed in 3 ml of 0.9% NaCl sterile solution and disrupted in a glass tissue homogenizer. The number of viable mycobacteria was determined by plating homogenates on Middlebrook 7H10 agar (Difco) plates containing 10% Middlebrook OADC enrichment (Becton Dickinson). Lung samples from untreated mice were diluted 1:100, 1:1,000 and 1:10,000 (100 μl of each dilution was plated). For treated groups, we plated 100 μl and 500 μl of the crude lung homogenates (without dilutions) as well as 100 μl of 1:10-diluted lung samples. Spleen samples from untreated mice were diluted 1:100, 1:1,000 and 1:10,000 (100 μl of each dilution was plated). For treated animals, we plated 100 μl from the crude spleen homogenates (without dilutions) as well as 100 μl from diluted 1:10 and 1:100 spleen samples. Plates were incubated at 37 °C for 4 weeks in a 5% CO_2 environment prior to the counting of viable *M. tuberculosis* cells. CFU numbers were converted to logarithms of CFU (log_{10} CFU) and data were evaluated by one-way analysis of variance (ANOVA), followed by Bonferroni's post-test, using GraphPad Prism 5.0 (San Diego, CA, USA). Differences were considered significant at the 95% level of confidence.

2.2. CYP450 inhibition assay

To evaluate the potential of IQG-607 to inhibit CYP450 enzymes, the compound was incubated with pooled human liver microsomes in combination with specific probe substrate for each CYP450 isoform tested (1A2, 2C9, 2C19, 2D6, 2E1, 3A4), according to previously described protocols (Yan and Caldwell, 2004; Muradás et al., 2018). The enzyme inhibition assays were also performed using isoform-selective inhibitor for each CYP450 isoform. NADPH was added to reaction mixture to supply electrons for enzymatic CYP450 reactions and the formation of metabolite from each substrate was monitored by HPLC MS/MS techniques. The concentration of IQG-607 that causes 50% reduction of the activity of each CYP450 isoform (IC_{50}) was determined. The inhibitors, probe substrates and its monitored metabolites for each isoform are summarized in Table 1. These experiments were carried out by the company CEMSA (Centro de Espectrometria de Massa Aplicada, São Paulo, Brazil) as service rendering.

Table 1
CYP450 inhibition investigation in human liver microsomes.

CYP isoform	Probe substrate	Metabolite	Selective inhibitor (concentration range tested, μM)	Incubation time (min)	Extractor solvent	Inhibitor IC_{50}[a] (μM)	IQG-607 IC_{50}[a] (μM)
CYP1A2	Phenacetin	Acetaminophen	Naphthoflavone (1–10)	30	Ethyl acetate	0.015	> 30
CYP2C9	Tolbutamide	4′-Hydroxytolbutamide	*Sulfaphenazole (0.1 - 5)*	20	Ethyl acetate	0.6	> 30
CYP2C19	S-Mephenytoin	4′-Hydroxymephenytoin	Ticlopidine (1–30)	45	Acetonitrile	6.4	> 30
CYP2D6	Dextromethorphan	Dextrorphan	Quinidine (0.4–30)	15	Ethyl acetate	0.04	> 30
CYP2E1	Chlorzoxazone	6-Hydroxychlorzoxazone	4-methylpyrazole (7.3–72.3)	30	Ethyl acetate	0.02	20
CYP3A4/5	Midazolam	1′-Hydroxymidazolam	Ketoconazole (0.01–0.25)	8	Ethyl acetate	0.08	> 30

[a] IC_{50} values were estimated by a nonlinear regression of the percentage of remaining enzyme activity versus the concentration of IQG-607 or specific inhibitor. The maximum concentration of IQG-607 tested for all isoforms was 30 μM.
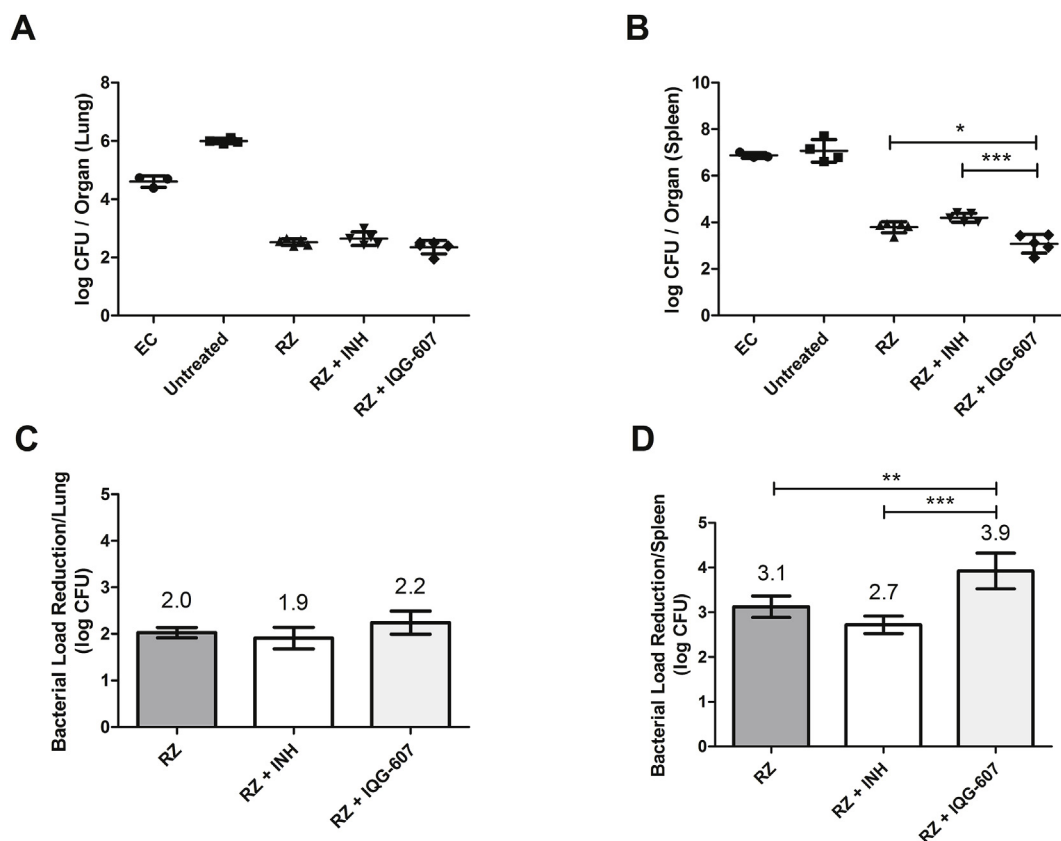
Fig. 1. Effects of IQG-607 combined with first-line anti-tuberculosis drugs. CFU counts in lungs (**A**) and spleens (**B**) from *M. tuberculosis*-infected mice after treatment with rifampin plus pyrazinamide (RZ), rifampin plus pyrazinamide plus isoniazid (RZ + INH), or rifampin plus pyrazinamide plus IQG-607 (RZ + IQG-607). Results represent the means ± SD of 4–6 mice per group. Early control (EC) represents mice sacrificed at the start of treatment and "Untreated" group represents mice sacrificed at the end of the treatment. Bacterial load reductions, defined as the differences between bacterial loads after treatments and EC, in lungs (**C**) and spleens (**D**), are shown. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ compared to IQG-607-containing group. Data were evaluated by ANOVA, followed by Bonferroni post-test, using GraphPad Prism 5.0.

2.3. Toxicity assays

2.3.1. Salmonella/microsome mutagenicity test

Mutagenicity was evaluated using the pre-incubation procedure as reviewed in previous studies (Maron and Ames, 1983; Mortelmans and Zeiger, 2000). *Salmonella typhimurium* strains TA98, TA97a, TA100, TA102, and TA1535 were provided by MOLTOX (Molecular Toxicology Inc., USA). IQG-607 was dissolved in sterile distilled water and concentrations used were determined according to a range finder experiment in TA100 strain, with and without metabolization, and cytotoxicity was observed at concentrations higher than 400 µg/plate (data not shown). Hence, the concentrations used were 10, 50, 100, 200, and 400 µg/plate. IQG-607 was dissolved in sterile distilled water and concentrations used were determined according to a range finder experiment in TA100 strain, with and without metabolization, and were 10, 50, 100, 200, and 400 µg/plate. Briefly, 100 µl of test bacterial cultures (1–2 x 10^9 cells/mL) were incubated at 37 °C with different amounts of IQG-607 in the presence or absence of S9 mix (4% S9 fraction) for 20 min, without shaking. Subsequently, 2 ml of soft agar (0.6% agar, 0.5% NaCl, 50 µM histidine, 50 µM biotin, pH 7.4, 42 °C) were added to the test tube and poured immediately onto a plate of minimal agar (1.5% agar, Vogel-Bonner E medium, containing 2% glucose). 2-aminoanthracene (2-AA, 5 µg/plate) was used as positive control for all strains in the presence of metabolic activation (with S9 mix). In the absence of metabolic activation, 4-nitroquinoline-oxide (4-NQO, 0.5 µg/plate) was used for TA98, TA97a, and TA102 strains, while sodium azide (NaN₃, 1 µg/plate) was employed for TA100 and TA1535 strains. Plates were incubated in the dark at 37 °C for 48 h before counting the

revertant colonies. Assays were repeated twice and plating for each dose was carried out in triplicates.

Results are expressed as means ± S.D. and statistical significance was determined by ANOVA, followed by Dunnett's test. In all comparisons, $P < 0.05$ was considered as indicative for statistical significance. A test substance was considered mutagenic in the *Salmonella*/microsome assay when significant ANOVA variance was observed, and the mean number of revertant on test plates was at least twice as high as that observed in the negative control plates (or at least three times higher, for the TA1535 strain).

2.3.2. Micronucleus formation test

The micronucleus formation test was performed to evaluate possible *in vivo* genotoxic effects of IQG-607. This study was conducted according to OECD 474 (OECD, 2016), *under Good Laboratory Practices (GLP) conditions*. Fifty male and female Swiss mice (from 6 to 10 weeks-old) were randomly assigned to 5 groups (5 males and 5 females per group). IQG-607 was administered *per os* at the dosages of 25, 100 or 250 mg/kg, for three consecutive days (24 h-interval between doses). Negative control animals received oral gavage with the vehicle, water. The positive control cyclophosphamide was given intraperitoneally at 25 mg/kg, for two consecutive days (Vasquez, 2010). After 18–24 h of the last dose, mice were euthanized, femurs removed, and the bone-marrow cells were flushed into tubes with 1 ml fetal bovine serum. The samples were centrifuged at 1,000 rpm for 5 min, and the pellet was used to prepare bone-marrow smear specimens. The specimens were dried at room temperature, fixed with methanol, and stained with Giemsa solution (Laborclin, Brazil), following instructions from the

manufacturer. Each sample received a different code and were randomized analyzed by an experienced observer blinded to treatment or control groups (OECD, 2016).

We first determined the ratios of immature (polychromatic erythrocytes, PCE) and mature (normo-chromatic erythrocytes, NCE) by counting 1,000 erythrocytes from each mouse bone marrow. The frequency of micronucleated polychromatic erythrocytes (MNPCE) was calculated from observations of 4,000 PCE from each animal. Data were statistically analyzed using ANOVA, followed by Bonferroni's post-test. These experiments were carried out by the company CIEnP (Centro de Inovação e Ensaios Pré-clínicos, Florianópolis, Brazil) as service rendering. This experimental protocol was approved by Animal Use and Care Committee from CIEnP (number 210). *The test substance, IQG-607, was synthesized and characterized according to* Oliveira et al. (2006) *by SSK Biosciences Pvt. Ltd. (Hyderabad, India), under Good Laboratory Practices conditions.*

3. Results and discussion

3.1. IQG-607 displays additive effects in combination with rifampin plus pyrazinamide (RZ)

Firstly, spleen weights were determined and no difference was found between treated groups, RZ, RZ + INH, and RZ + IQG-607 (data not shown). All groups that received the drugs in combination, RZ, RZ + INH or RZ + IQG-607, had their bacterial loads significantly reduced in both lungs (Fig. 1A) and spleens (Fig. 1B), when compared to EC and Untreated controls (***$P < 0.001$ for all comparisons, in both organs). When IQG-607 was administered in combination with RZ, CFU counts were lower (*$P < 0.05$) than those obtained with treatment of RZ alone, in spleens (Fig. 1B). This finding demonstrates that IQG-607 had additive effects when combined with the first-line anti-tuberculosis drugs rifampin and pyrazinamide in mice. Moreover, IQG-607-containing therapy caused an additional 10% bacterial burden reduction in lungs, compared to the RZ group (Fig. 1C), suggesting an additive effect for IQG-607 in lungs. Possibly due to different organ specific pharmacokinetic parameters for IQG-607, intensities of additive effects were different in lungs and spleens. At any rate, no antagonistic effect was observed when IQG-607 was added to the therapy.

Interestingly, the combination RZ + IQG-607 was more potent than RZ + INH (***$P < 0.001$), as evidenced in spleens (Fig. 1B and D). In the lungs, the IQG-607-containing combination caused an additional 15% CFU reduction, comparing to RZ + INH (Fig. 1C). We previously reported the anti-mycobacterial effects of IQG-607 orally administered as monotherapy in mice (Rodrigues-Junior et al., 2012). According to this previous study, the same dose (25 mg/kg) of INH or IQG-607, both given daily as monotherapies by oral route for 4 weeks to mice, resulted in similar effects in spleens and a modest increased effect of INH as compared to IQG-607 in the lungs (Rodrigues-Junior et al., 2012). These observations allow us to suggest the higher potency of RZ + IQG-607 compared to RZ + INH in the mouse model of tuberculosis infection. Moreover, we found that INH does not enhance the therapeutic activity of rifampin plus pyrazinamide in mice, which is consistent with previous observations (Grosset et al., 1992; Almeida et al., 2009).

3.2. IQG-607 has low to moderate inhibitory activity against CYP450 isoforms

CYP450 enzymes are responsible for the biotransformation of many drugs (Yan and Caldwell, 2004; Kerns and Di, 2008). Since anti-tuberculosis drugs have been used as combined regimens in human chemotherapy, inhibition of CYP450 isoforms could cause important drug-drug interactions and adverse effects. Therefore, we investigated the inhibitory potential of IQG-607 against a panel of human CYP450 isoforms (3A4, 1A2, 2C9, 2C19, 2D6, and 2E1). Results showed that IQG-607 presented weak to moderate inhibitory activity against CYP450, as

the IC_{50} values were > 20 μM for all isoforms (Table 1). According to the literature, during *in vitro* CYP450 inhibition assessment, an IC_{50} value larger than 10 μM is suggestive of low or moderate inhibitory potential (Kerns and Di, 2008).

INH was previously suggested as a potential inhibitor of CYP2C19 and CYP3A CYP450 isoforms in human liver microsomes in concentrations found in patients (Desta et al., 2001; Wen et al., 2002). These observations could explain, at least in part, the mechanism by which INH slows the elimination of co-administered drugs, including phenytoin, carbamazepine, and diazepam (Desta et al., 2001). This profile confers further implication and importance for slow acetylators of INH, in which INH concentrations are higher, leading to a greater risk for adverse drug interactions (Desta et al., 2001). Considering that CYP3A metabolizes 50% of drugs and CYP2C19 metabolizes 2% (Kerns and Di, 2008), it would be beneficial if INH was replaced with a non-inhibitor of CYP3A isoform.

Based on *in vivo* oral pharmacokinetic data available for IQG-607 to date (Dadda et al., 2018), the compound concentration found in mice (3.85 μM or 1.72 μg/ml) is lower than the IC_{50} values estimated for all CYP450 isoforms analyzed. Moreover, fortunately, IQG-607 did not inhibit or weakly inhibited three important isoforms, CYP3A4, CYP2C9, and CYP2C19, which account for most of cytochrome metabolic system (Yan and Caldwell, 2004; Kerns and Di, 2008). Taken together, these findings suggest a reduced risk of drug − drug interactions for IQG-607 and thus warrant further *in vivo* investigations.

3.3. Favorable safety profile for IQG-607 in vitro and in vivo

The *Salmonella*/microsome assay showed IQG-607 induced base pair substitution mutations, as observed by increasing his^+ revertant colonies significantly with MI higher than 2 in TA100 and higher than 3 in TA1535, in the absence of S9 mix (Table 2). However, there was no mutagenic induction with the strains detecting frameshift mutations TA98 and TA97a. Moreover, IQG-607 showed negative result with TA102 strain that detects oxidative, alkylating, and crosslinking agents. Aiming to mimic human metabolic processes, we included incubation with S9 mix, and no mutagenic effect was detected with any strain, indicating that IQG-607 was possibly metabolized to non-mutagenic chemical compounds. In TA1535, the higher concentration tested showed significant increase in his^+ revertant colonies, but the MI was lower than 3, and the result was considered negative (Table 2). Similarly to IQG-607, INH was found to have mutagenic effects in the repair-deficient strains TA1535 and TA100, without metabolic activation (Braun et al., 1984). Furthermore, INH was considered a promutagen molecule in the *S. typhimurium* system, since after its incubation with crude mammalian liver homogenates, mutagenic effects were observed, independently of the repair apparatus of the tested cells (Braun et al., 1984).

Additionally, we aimed at investigating the genotoxic potential of IQG-607 in the *in vivo* micronucleus assay. This non-clinical test was performed following GLP conditions (Andrade et al., 2016), since the data obtained here might be required in the subsequent phases of the drug development process. The first finding was that IQG-607 or cyclophosphamide, used as positive control drug, did not alter the ratios of immature and mature erythrocytes (PCE/NCE) in bone marrow, compared to untreated controls (Fig. 2A). Cyclophosphamide caused a statistically significant increase in micronucleus formation, compared to controls (Fig. 2B). Moreover, IQG-607 did not increase the MCPCE frequencies at any dose tested, 25, 100, or 250 mg/kg (Fig. 2B). It is important to mention that these doses were chosen based on previous toxicity and efficacy data obtained for rodents (Basso et al., 2010; Rodrigues-Junior et al., 2012, 2017a).

IQG-607 did not show any genotoxic potential in the mouse micronucleus assay, and these findings are in agreement with previous report showing lack of genotoxic effects for IQG-607 in the comet assay (Amorim et al., 2017). It was observed that pulmonary tuberculosis

Table 2

Induction of his^+ revertants in *S. typhimurium* strains by IQG-607 with and without metabolic activation (S9 mix).

Substance	Concentration (µg/plate)	TA98 Rev/plate[a]	MI[b]	TA97a Rev/plate[a]	MI[b]	TA100 Rev/plate[a]	MI[b]	TA1535 Rev/plate[a]	MI[b]	TA102 Rev/plate[a]	MI[b]
S. typhimurium strains											
Without metabolic activation (-S9)											
NC[c]	–	19.0 ± 3.6	-	86.7 ± 26.7	-	92.7 ± 12.0	-	10.3 ± 2.1	-	313.7 ± 35.2	-
IQG-607	10	16.7 ± 1.5	0.88	87.3 ± 20.3	1.01	139.0 ± 21.7	1.50	20.3 ± 1.2	1.97	306.7 ± 6.8	0.98
	50	19.0 ± 6.2	1.00	90.0 ± 9.5	1.04	283.3 ± 19.0***	3.06	60.7 ± 13.4*	5.87	297.0 ± 36.4	0.95
	100	20.0 ± 5.6	1.05	101.0 ± 14.7	1.17	335.3 ± 47.6***	3.62	99.7 ± 6.7***	9.65	320.0 ± 42.7	1.02
	200	18.0 ± 3.6	0.95	96.7 ± 15.5	1.12	379.3 ± 30.9***	4.09	151.3 ± 40.8***	14.65	268.7 ± 28.2	0.86
	400	13.7 ± 7.0	0.72	57.7 ± 29.6	0.67	264.3 ± 79.1**	2.85	239.7 ± 14.6***	23.20	237.7 ± 40.7	0.76
PC[d]	0.5 (4NQO) 1 (NaN₃)	149.0 ± 34.6***	7.84	601.0 ± 128.7***	6.93	1357.0 ± 280.1***	14.64	658.5 ± 142.1***	63.75	1065.0 ± 77.1***	3.39
With metabolic activation (+S9)											
NC[c]	–	28.7 ± 8.1	-	75.7 ± 6.8	-	102.0 ± 8.7	-	8.3 ± 2.1	-	319.0 ± 40.7	-
IQG-607	10	27.0 ± 4.4	0.94	94.0 ± 20.0	1.24	105.3 ± 5.9	1.03	10.3 ± 2.3	1.24	363.3 ± 34.1	1.14
	50	28.0 ± 5.7	0.98	97.0 ± 42.0	1.28	99.0 ± 17.4	0.97	13.3 ± 4.0	1.60	362.7 ± 2.3	1.14
	100	27.7 ± 3.8	0.97	79.0 ± 16.8	1.04	118.0 ± 6.0	1.16	8.7 ± 2.3	1.04	361.7 ± 36.2	1.13
	200	20.0 ± 1.0	0.70	63.3 ± 26.6	0.84	111.3 ± 6.8	1.09	10.0 ± 3.6	1.20	366.3 ± 10.6	1.15
	400	19.0 ± 11.0	0.66	116.7 ± 36.7	1.54	121.0 ± 15.7	1.19	23.0 ± 7.8**	2.76	352.7 ± 38.8	1.11
PC[d]	5 (2-AA)	1740.0 ± 237.3***	60.69	1152.0 ± 67.9***	15.22	1243.0 ± 60.8***	12.19	57.7 ± 7.5***	6.92	1470.0 ± 12.73***	4.61

*p < 0.05.

**p < 0.01.

***p < 0.001 (ANOVA, Dunnett's test).

[a] Number of revertants/plate (Rev/plate): mean of three independent experiments ± SD.

[b] MI: mutagenic index (number of his^+ induced in the sample/number of spontaneous $his+$ in the negative control).

[c] NC: negative control (100 µL distillated water used as solvent for IQG-607).

[d] PC: positive control (-S9) sodium azide to TA100 and TA1535; 4-nitroquinoline-oxide to TA97a, TA98 and TA102; (+S9) 2-aminoanthracene; Significantly different compared to the negative control.

patients possess higher frequencies in chromosomal aberrations and micronuclei formation compared to healthy controls (Masjedi et al., 2000). In addition, the combined therapy with anti-tuberculosis drugs (INH plus rifampin plus pyrazinamide plus ethambutol or streptomycin) was suggested to cause genotoxicity in both humans and rodents (Masjedi et al., 2000; Arslan et al., 2015). In this context, it would not be reasonable to include other potentially genotoxic drug to the therapeutic scheme, since it could cause additional toxic effects.

4. Conclusion

The World Health Organization warns for a serious lack of new antibiotics under development to combat persisting infectious diseases, such tuberculosis. New antibiotics must be urgently discovered and developed. Ideally, a new anti-tuberculosis drug should diminish the length of treatment, lower the dosing frequency, and have lower toxicity issues (Pai et al., 2016; WHO, 2018). Aiming to develop a new oral drug to treat tuberculosis, IQG-607 has been studied for more than fifteen years (Abbadi et al., 2018). Special attention must be taken with drug toxicity and safety pharmacology, mandatory non-clinical studies which must be performed before regulatory applications (Andrade et al., 2016). This study revealed favorable toxicological and efficacy results for IQG-607 based on results obtained by different experimental approaches.

In combination with other anti-tuberculosis drugs in animals, IQG-607 demonstrated additive effects when combined with rifampin plus
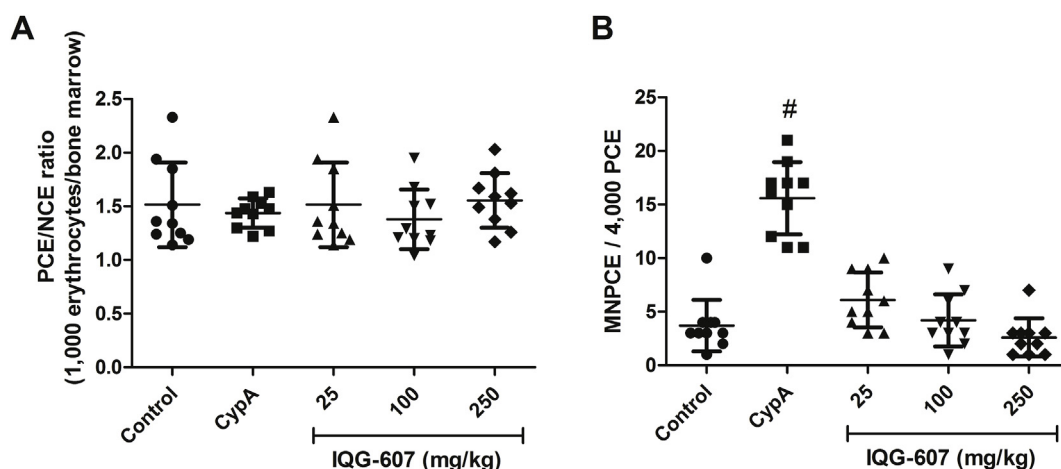

Fig. 2. *In vivo* mouse bone marrow micronucleus formation. Ratio of polychromatic and normochromatic erythrocytes (PCE/NCE) in 1,000 erythrocytes from each mouse bone marrow (**A**). Micronucleated polychromatic erythrocytes (MNPCE) found in 4,000 immature erythrocytes per mouse (**B**). Results represent the means ± SD of 10 mice (5 male and 5 female) per group. #P < 0.001 compared to negative control group ("Control"). Data were evaluated by ANOVA, followed by Bonferroni post-test. "CypA" represents cyclophosphamide-treated group (positive control), and 25, 100, or 250 represent IQG-607 doses, in mg/kg.

pyrazinamide. It is tempting to infer that this combination might be an interesting alternative to develop more effective therapeutic schemes, that might reduce the treatment duration, for humans. In addition, IQG-607 did not display any mutagenic or genotoxic effects, confirming the favorable safety profiles previously observed in cells (Amorim et al., 2017), rodents (Basso et al., 2010; Rodrigues-Junior et al., 2017a) and non-rodents. (Rodrigues-Junior et al., 2017b).

Additionally, IQG-607 showed low to moderate inhibitory potential against human CYP450 isoforms (3A4, 1A2, 2C9, 2C19, 2D6, and 2E1), suggesting a reduced risk for drug-drug interactions and adverse effects, when administered in drug combinations. Taken together, all toxicological data available to date suggest that IQG-607 could be considered as a safe option to replace INH, for example. Furthermore, IQG-607 could be proposed as a substitute for INH in clinical tests aiming to reduce treatment duration.

In summary, IQG-607 promising *in vivo* activity and toxicological data suggest that IQG-607 is a candidate for clinical development as a new anti-tuberculosis drug. In this regard, we are currently studying a suitable formulation to administer IQG-607 and planning phase I clinical studies to evaluate IQG-607 safety and bioavailability in healthy humans.

Declaration

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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