Nonclinical safety, tolerance and pharmacodynamics evaluation for meplazumab treating chloroquine-resistant *Plasmodium falciparum*

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Abstract Meplazumab is an anti-CD147 humanized IgG2 antibody. The purpose of this study was to characterize the nonclinical safety, tolerance and efficacy evaluation of meplazumab treating chloroquine resistant *Plasmodium falciparum*. Meplazumab was well tolerated in repeat-dose toxicology studies in cynomolgus monkeys. No observed adverse effect level was 12 mg/kg. No difference between genders in the primary toxicokinetic parameters after repeat intravenous injection of meplazumab. No increased levels of drug exposure and drug accumulation were observed in different gender and dose groups. Meplazumab had a low cross-reactivity rate in various tissues and did not cause hemolysis or aggregation of red blood cells. The biodistribution and excretion results indicated that meplazumab was mainly distributed in the plasma, whole blood, and hemocytes, and excreted in the urine. Moreover, meplazumab...
1. Introduction

Plasmodium falciparum malaria is a serious threat to worldwide healthcare. To date, hundreds of millions of malaria cases occur worldwide, most of which are caused by *P. falciparum* 1. Chemical drugs have been used for the elimination of *P. falciparum*, such as artemisinin and its derivatives 2–4. As a good antimalarial drug, chloroquine soon lost its therapeutic effect on *P. falciparum* after the emergence of resistant strains 5–8. Chloroquine has been removed from first-line antimalarial for the elimination of *P. falciparum* by World Health Organization (WHO). In areas with chloroquine-susceptible infections, chloroquine is indicated for the treatment of uncomplicated *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* or *Plasmodium knowlesi* malaria 1. A large number of studies have shown that chloroquine resistance is strongly associated with mutations of the *Pfcrt* gene, which encodes the chloroquine resistance transporter to participate in efflux chloroquine from the intracellular digestive vacuole, the site of drug action 9,10. *Pfmdr1* gene mutation is also related to the production of chloroquine resistance 11–13. Artemisinin-based combination therapy has become the standard antimalarial therapy recommended by the WHO, and is considered to be the most effective treatment for severe malaria 14. It is disappointing that the emergence of artemisinin-resistant strains is a serious challenge for artemisinin-based combination therapy 15. New antimalarial drugs are urgently needed for malaria elimination campaigns. Targeted biological drugs are becoming a new direction of malaria drug research and development. Antibodies expressed on the surface of merozoites are considered blood stage malarial vaccine candidates, some of which are in clinical testing, e.g., reticulocyte-binding protein homolog 5 (RH5), part of a trophozoite-exported protein (P27A), merozoite surface protein 3, and apical membrane antigen 1 16. Zhang et al. 17 previously identified a new pair of ligand–receptor proteins, i.e., rhoptry-associated protein 2 (RAP2), a rhoptry protein expressed on the surface of merozoites, and its receptor CD147 on erythrocytes. Their interaction occurs in the parasitophorous vacuole formation stage of the invasion. Zhang et al. 18 demonstrated that HP6HS is also known as meplazumab, a humanized CD147 monoclonal antibody (mAb), exhibited excellent efficacy in the treatment and prevention of severe malaria in humanized mice, and it is believed that it would become a novel targeted antimalarial drug in the blood stage. Compared with traditional chemical drugs, monoclonal antibodies have obvious advantages, including high specificity, low toxicity, long half-life in the blood, etc. In the treatment of diseases, both the variable region (Fab) and crystalline region (Fc) of mAbs can play an important role. The Fab segment of mAbs can perform its biological function by identifying free targets in the blood circulation or by targeting proteins on the cell surface 20,21. The Fc segment of mAbs can enhance the immune defense mechanism by antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cytophagy, and complement dependent cytotoxicity effects 22–24. Zhang et al. 19 have demonstrated that the Fab segment of meplazumab blocked the invasion of *P. falciparum* by targeting CD147 on the erythrocyte surface.

Detailed preclinical pharmacokinetic and toxicology data of mAbs determine whether they can be used in human clinical trials 25. The pharmacokinetic study of mAbs can provide us with data on the tissue distribution, metabolic pathway, and retention time in vivo. Traditional non-antibody drugs have a half-life of only a few hours, while mAbs have a serum/plasma half-life of several days or more in humans. Parenteral administration, slow tissue distribution, and a long elimination half-life are the most pronounced clinical pharmacokinetic characteristics of mAbs. Monoclonal antibodies have gained distinct competitive advantages in the field of biopharmaceuticals. However, several mAbs have been discontinued or withdrawn based either on their inability to demonstrate efficacy and/or due to adverse effects, e.g., efalizumab 26. Furthermore, even some of the mainstream anti-cancer mAb drugs have had some adverse events reported. These adverse events have mainly manifested as toxic and side effects to different organ systems during the administration of mAbs, including anemia, allergic reactions, gastrointestinal toxicity, neurotoxicity, cardiotoxicity, etc. 27–29. The association between these adverse events and the mAbs remains to be confirmed, but it still demonstrates the importance of preclinical toxicology studies. Preclinical toxicology studies of mAbs allow people to predict and handle the potential side effects and clinical toxicity in humans. Suitable animal models are very important for the study of preclinical pharmacokinetics and toxicology of mAbs 30. Animal models should have similar pharmacology, pharmacokinetic and toxicology profiles to humans 31–33. At present, Sprague–Dawley (SD) rats, pigs, dogs, and non-human primates are widely used in preclinical studies of mAbs. More nonclinical safety and tolerance evaluations of meplazumab are needed to support its clinical trials.

CD147 is a highly glycosylated type 1 single transmembrane glycoprotein, belonging to the immunoglobulin superfamily. CD147 is an important adhesion molecule and plays a significant role in numerous pathological processes, including cancer, inflammation, atherosclerosis, and ischemic myocardial injury. 34 CD147 is expressed on erythrocyte lineage cells throughout erythropoietic development, including mature erythrocytes, and is the target for *Plasmodium* merozoites to allow reorientation and subsequent invasion of erythrocytes. Specifically, CD147 interacts with RH5 to facilitate the relocation of *P. falciparum* on the surface of erythrocytes 35,36, and with the parasite ligand RAP2 to...
promote the formation of the *P. falciparum* parasitophorous vacuole. As meplazumab binds to CD147 expressed on the human erythrocyte cell surface through the complementary determining region, it directly blocks the CD147–RAP2 interaction and the formation of the parasitophorous vacuoles needed for *P. falciparum* invasion of human erythrocytes. This is a different mechanism from other anti-falciparum malaria drugs. Zhang et al. found that the CD147–RAP2 interaction is essential for *P. falciparum* to invade human erythrocytes and confirmed that the anti-CD147 mAb meplazumab can effectively treat and prevent *P. falciparum* infection in vivo. The efficacy against chloroquine-resistant *P. falciparum* infection of meplazumab in vivo is still unclear.

In this study, we demonstrated that meplazumab was safe and tolerated in cynomolgus monkeys and SD rats. In addition, within the given concentration range, meplazumab had a low cross-reactivity rate in various tissues and did not cause hemolysis or aggregation of red blood cells (RBCs). Finally, we evaluated the efficacy against chloroquine-resistant *P. falciparum* infection of meplazumab in a humanized mouse model. Taken together, the nonclinical data provided by this study are an important basis for the clinical research and application of meplazumab.

2. Materials and methods

2.1. Cell lines and cell culture

The human lung cancer cell line A549 was obtained from American Type Culture Collection (Manassas, VA, USA). After expansion, this cell line was deposited into the National Platform of Experimental Cell Resources for Sci-Tech (Beijing, China). This cell line was subjected to short tandem repeat tests and authenticated before utilization for researches. This cell line was maintained in high-glucose DMEM supplemented with 10% fetal calf serum at 37 °C and 5% CO2 in a humidified incubator.

2.2. Meplazumab

Meplazumab is a recombinant, humanization IgG2 antibody constructed by complementary determining region-grafting, directed against the extracellular region of human CD147. Meplazumab is produced by cell culture in bioreactors, using serum-free media at Pacific Meinuoke Biopharmaceutical Co., Ltd. (Changzhou, China). After purification, the mAb was resolved in histidine/histidine hydrochloride buffer solution (pH 7.2) containing polysorbate 80 and sucrose, vialled under aseptic conditions, and formulated as a colorless, sterile product without any preservatives.

2.3. Reagents

Metuzumab was supplied by Pacific Meinuoke Biopharmaceutical Co., Ltd. RPMI medium 1640 (72,400-047) was purchased from Gibco (Grand Island, NY, USA). Hypoxanthine (H9377-5G) and chloroquine (C6628-25G) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Clodronate liposomes were purchased from Liposoma B.V. (Amsterdam, The Netherlands). IgG from human serum was purchased from Genia (Beijing, China). Anti-human CD235a PE antibody (12-9987-82, RRID: AB_466,300) was purchased from Thermo-Fisher (Waltham, MA, USA). CD147-his protein and anti-meplazumab polyclone antibody were supplied by Fourth Military Medical University (Xi’an, China). Human IgG-heavy and light chain monkey-adsorbed antibody conjugate horseradish peroxidase (HRP) was purchased from Bethyl (Montgomery, AL, USA). 125I-Meplazumab was supplied by MITRO Biotech Co., Ltd. (Nanjing, China). The CD31 antibody (11265-1-AP) was purchased from Proteintech Group, Inc. (Wuhan, China).

2.4. Animals

NOG (NOD/Shi-scid/IL-2Rγ null, female, 12 weeks old) mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All experiments were performed under specific-pathogen-free conditions and obeyed the experimental animal protocols that were approved by the Laboratory Animal Welfare and Ethics Committee of Medical Department of Fourth Military Medical University (Xi’an, China).

SD rats (half of each sex, 180–220 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. SD rats were received according to the standard operating procedures of MITRO Biotech Co., Ltd. (Nanjing, China), and the experimental animals were housed in the barrier facility for specific-pathogen-free animals. Experimental animal use permission was SYXK (Su) 2015–0014.

Cynomolgus monkeys (half of each sex, 2.8–4.7 kg) for toxicology studies were provided by Guangdong Chunsheng Biological Technology Development Co., Ltd. (Guangzhou, China). The cynomolgus monkeys were chosen as the non-rodent species for drug safety evaluation because it is accepted by health authorities worldwide as an appropriate experiential animal model. Meanwhile, since the test article is a humanized monoclonal antibody, cynomolgus monkeys are very sensitive to it. The toxicology studies were conducted in compliance with the “Guide for the Care and Use of Laboratory Animals” (2011) issued by the National Research Council, USA, and the “Laboratory Animal Administration” (2nd edition, 1988) issued by the State Science and Technology Committee, China.

2.5. Surface plasmon resonance measurements

The affinity (Kd) for the binding of human and cynomolgus monkey CD147 antigens to meplazumab was measured on a multi-SPR array system (ProteOn XPR36, Bio-Rad, Hercules, CA, USA). Briefly, meplazumab (10 μg/mL) was fixed to the chip, and various concentrations of CD147 extracellular protein from humans and cynomolgus monkeys (0, 0.625, 1.25, 2.5, 5, and 10 nmol/L) were injected into different channels. After 3 min of binding and 12 min of dissociation at a flow rate of 50 μL/min, the obtained data were analyzed using the Langmuir model (1:1).

2.6. Flow cytometry assay

Levels of CD147 expression on erythrocytes from different species were evaluated using flow cytometry. RBCs from humans (7 cases) and cynomolgus monkeys (10 cases) were incubated with meplazumab (4 or 40 μg/mL) at 37 °C for 1 h. An anti-human IgG antibody was incubated at 4 °C for 1 h. After rinsing twice, the results were detected using flow cytometry.
2.7. A 4-week repeat-dose intravenous toxicity study of meplazumab in cynomolgus monkeys with an 8-week recovery period

A total of 40 cynomolgus monkeys were assigned into four groups: vehicle control group (solvent of meplazumab for injection: 0 mg/kg/day), and meplazumab groups at low (2 mg/kg/day), middle (6 mg/kg/day), and high (12 mg/kg/day) doses. The dose volume was 5 mL/kg. Each group consisted of 10 animals (half of each sex). Animals were dosed once weekly (Days 1, 8, 15 and 22, respectively). Necropsy was performed in 6 animals (half of each sex) at the end of the dosing period and the remaining 4 animals (half of each sex) at the end of the recovery period. Tissues/organs were collected and processed for histopathological examination. The toxicological endpoints evaluated during the dosing and recovery periods included the following: mortality/moribundity observations, clinical observations, dose site irritation observation, body weight, food consumption, rectal temperature, electrocardiogram (lead II), blood pressure, respiratory rate, ophthalmology examination, hematology/coagulation/clinical chemistry/immune function, urinalysis, occult blood analysis in feces, and bone marrow smear examination.

Blood samples were drawn from the femoral veins on the opposite side of the injection sites at different time points from all animals for toxicokinetic analysis. For the vehicle group, blood samples were collected pre-dosing and 4 h post dosing. For meplazumab groups, during the first dosing, blood samples were collected pre-dosing and at 0.25, 4, 24, 48, 72, 96 and 168 h (pre-dosing on Day 8) post-dosing; during the fourth dosing, blood samples were collected pre-dosing and at 0.25, 4, 24, 48, 72, 96, and 168 h post-dosing. All blood samples were collected into the tubes containing separation gel and coagulant, and kept on crushed ice. The collected blood samples were centrifuged at approximately 3500 rpm (Beckman, Brea, CA, USA) for 5 min at 4 °C. After centrifugation, the serum samples were separated and stored at −80 °C until further analysis.

Serum concentration of meplazumab will be determined using a validated ELISA method. Briefly, for the quantification of meplazumab in serum samples, 96-well-microplates were coated with CD147-his coating solution. Meplazumab in the samples were combined with antibody-coated plate, then human IgG-heavy and light chain monkey-adsorbed antibody conjugate HRP was added. Responses were developed using tetramethylbenzidine as substrate, and the reaction was stopped with H2SO4 solution. The absorbance at 450 nm/630 nm was then read by Microplate Reader (Epoch, BioTek Instruments, Inc., Winooski, VT, USA).

Toxicokinetic parameters, including (if applicable) but not limited to Cmax, Tmax, and AUC0–168 h, were calculated using Watson 7.4 software (Thermo-Fisher).

2.8. Anti-drug antibody (ADA) assays

In toxicology study, all surviving animals were submitted for immunogenicity test. Serum samples were collected for the immunogenicity analysis at acclimation period (once), Days 14 and 28 (dosing period), 42, 56, 70, and 84 (recovery period).

ADA in cynomolgus monkey serum following injection of meplazumab was detected using validated bridging ELISA methods. Briefly, microplates were coated with meplazumab, incubated at 2–8 °C overnight, and then blocked with 5% skimmed milk powder for at least 2 h. Samples were diluted 20 times with 1% albumin from bovine serum and added to each well. Biotin-labeled meplazumab was then added. The “test item-ADA-test item” complex formed was detected with the streptavidin-HRP in combination with tetramethylbenzidine substrate. The chromogenic reaction was stopped with stop solution, and the signal at 450 nm/560 nm was recorded by Microplate Reader (Epoch, BioTek Instruments, Inc.). The intensity of the signal was positively correlated with the concentration of the ADA for meplazumab.

2.9. In vitro hemolysis studies

New Zealand white rabbits are internationally accepted non-rodent species for assessment of the hemolysis test of pharmaceuticals. One New Zealand white rabbit (female, 3.2 kg) was purchased from Chinese Food and Drug Inspection Institute (Beijing, China) and housed in the barrier facility for specific-pathogen-free animals. Experimental animals use license number is SYXX (Hu) 2014-0009. Cynomolgus monkeys were chosen to evaluate the safety of meplazumab in vivo in this study. Therefore, in vitro hemolysis studies, the blood from New Zealand white rabbit and cynomolgus monkey were used to assess the potential hemolysis test. In addition, the maximal intended clinical concentration of meplazumab was 2 mg/mL. The operation was as follows. The whole blood collected from a New Zealand white rabbit and a cynomolgus monkey was gently shaken for 10 min using flash with beadings for removing fibrin. RBCs were isolated after centrifugation (Beckman, 1500 rpm for 15 min) using an appropriate amount of 0.9% sodium chloride injection at room temperature. The above procedures were repeated 2 times until transparent and colorless supernatants were obtained. A 2% RBC suspension was prepared by diluting with 0.9% sodium chloride injection. The groups were divided into negative control (0.9% sodium chloride injection), vehicle control, positive control (deionized water), and meplazumab (different concentrations, the max concentration up to 2 mg/mL) groups. The RBCs of New Zealand white rabbit and cynomolgus monkey were mixed with test article or control. The character of solution was observed at 15, 30, 45, 60, 120 and 180 min after incubation at 37 °C. The solution showed red transparent indicating hemolysis. Brown-yellow or red-brown floccule deposition was observed in solution when RBCs aggregated and the solution did not disperse after vibration.

2.10. Tissue cross-reactivity studies

A tissue cross-reactivity study was performed on normal tissues from humans and cynomolgus monkeys by immunohistochemical staining. The normal human tissues were collected from the department of pathology, Xijing hospital (Xi’an, China). All individuals provided written informed consent, and the study was approved by the Hospital Ethics Committee (Xi’an, China).

In order to detect the binding ability, the test article was applied to formalin-fixed paraffin-embedded (FFPE) slices of normal human, and cynomolgus monkey tissues (3–8 donors per tissue) at two concentrations. Meplazumab at 1 μg/mL was selected as the lower concentration, and meplazumab at 20 μg/mL was selected as the high concentration. The blank control was 1 × phosphate buffered saline (PBS), and an anti-CD31 antibody directed against CD31, an endothelial cell biomarker, was used as an antigen preservation system control. Human lung adenosquamous carcinoma and hepatocellular carcinoma tissues were selected as the positive control tissues.
The FFPE slices were deparaffined and dehydrated following standard protocols. The antigens were retrieved by autoclaving in citric acid buffer (pH 6.0). Endogenous peroxidase was quenched by peroxidase blocking solution, and endogenous biotin was blocked by nonspecific staining blocker. After rinsing, the FFPE slices were incubated with meplazumab (1 or 20 μg/mL), rabbit anti-human/monkey CD31 polyclonal antibody or PBS, at 4°C overnight. After washing with PBS, the FFPE slices were incubated with biotinylated-coupled secondary antibodies according to the type of primary antibody for 15 min at room temperature. After incubation with peroxidase-labeled streptavidin, the FFPE slices were developed in 3,3-diaminobenzidine and counterstained with hematoxylin. The FFPE slices were dehydrated, mounted, and visualized under a light microscope. The expression level was evaluated by two senior pathologists.

2.11. Distribution and excretion in SD rats

Meplazumab was labeled with $^{125}$I through the iodogen method. The stability of the $^{125}$I-meplazumab labeled material was confirmed before injection. Trichloroacetic acid (TCA) precipitation was used to validate the methodology of $^{125}$I-meplazumab for the tissue distribution study.

Twenty-four SD rats (half of each sex) were administrated $^{125}$I-meplazumab at about 1 mg/kg or 20 ± 10 μCi per animal by a single intravenous injection. A group of 6 animals were sacrificed at 2, 96, 168, and 240 h post-administration. The urine, whole blood (preparation for whole blood, plasma, and hemocytes), heart, liver, spleen, lungs, kidneys, stomach, intestine, gonads, brain, fat, skeletal muscles, femurs, adrenal gland, thymus, mesenteric lymph nodes, and thyroid tissues were collected. The total radioactivity of the samples, as well as those after TCA precipitation, was measured using a gamma meter (Biodex, New York, NY, USA). Plasma and urine samples were collected from 1 female rat and 1 male rat at 2, 96, 168, and 240 h, and separated by high-performance liquid chromatography (HPLC, Biodex). The elution was measured using a gamma meter (Biodex), and diagrams were drawn using radioactivity versus time.

Six bile duct intubation model SD rats were administrated with $^{125}$I-meplazumab at approximately 1 mg/kg or 20 ± 10 μCi per animal by a single intravenous injection. Bile fluids were collected at 0–4, 4–8, 8–24, and 24–48 h post-administration. The total radioactivity of each sample was measured using a gamma meter (Biodex), and the cumulative recovery of radiolabeled samples excreted through the bile fluid path at each time point was calculated.

Six normal SD rats were administrated $^{125}$I-meplazumab at approximately 1 mg/kg or 20 ± 10 μCi per animal by a single intravenous injection. Urine and feces were collected at 0–8, 8–24, 24–48, 48–96, 96–168, 168–336, 336–504, 504–672, 672–840, 840–1008, and 1008–1176 h post-administration. The total radioactivity of each sample was measured using a gamma meter (Biodex). The cumulative percentage of radiolabeled samples excreted through the urine and feces paths at each time point were calculated.

2.12. ADCC assays

In vitro ADCC assays were performed as previously described. Briefly, the ADCC assays of meplazumab were performed using human peripheral blood mononuclear cells isolated from the blood of healthy humans (n = 2) as the effector cells, and the non-small-cell lung cancer A549 cells overexpressing CD147 as target cells. The target cells (1 × 10⁷ per well) were pre-incubated with different concentrations (12.5, 25, 50, 100, 200, 400, 600, 800, and 1000 μg/mL) of meplazumab or control antibody (metuzumab) for 30 min, prior to the addition of the effector cells at an effector/target ratio of 50:1. The cells were incubated for an additional 4 h and then the cell death was detected by measuring lactate dehydrogenase activity using a Cytotoxicity Detection Kit (Roche, Basel, Switzerland) according to the manufacturer’s protocols as in Eq. (1):

$$\text{Cytotoxicity (\%) = } \frac{\text{Experimental cell lysis} - \text{Spontaneous effector lysis} - \text{Spontaneous target lysis}}{\text{Maximum target lysis} - \text{Spontaneous target lysis}} \times 100$$  \hspace{1cm} (1)

All samples were assayed in triplicate wells in two independent experiments. The calculation of concentration for 50% of maximal effect was based on the four-parameter fitting curve.

2.13. Antimalarial efficacy of meplazumab in humanized mice

The establishment of a humanized mouse model infected with chloroquine-resistant strain referred to previous construction methods. Briefly, NOG mice were injected with 450 μL of type O human red blood cells (huRBCs), 250 μL of human serum from a type AB donor mixture via the inner canthus veniplex and 500 μL of a 20% clodronate liposomes mixture (100 μL of clodronate liposomes + 400 μL of RPMI 1640 + 50 mg/L hypoxanthine) by intraperitoneal injection three times a week. Subsequently, peripheral blood cells were collected via tail vein and incubated with anti-human CD235a PE (1:1200) for 40 min at room temperature. Samples were rinsed with PBS. The chimerism rate of the huRBCs was determined by flow cytometry (LSRFortessa™, BD Pharmingen, San Diego, CA, USA). Mice with chimerism rate of more than 30% were randomized into groups. The mouse models were divided into negative control (IgG from human serum, 5 mg/kg), positive control (chloroquine, 5 mg/kg), and meplazumab (5 mg/kg) groups. The cultured P. falciparum Dd2, which had not been synchronized, was used for infection when the parasite rate reached 1%. One hundred microliters of pure RBCs with a 1% parasite rate was mixed with 250 μL of human type AB serum. The mixture was injected via the inner canthus veniplex of mouse.

After infection, peripheral blood was sampled via the inner canthus veniplex of the mice to prepare a thin blood smear every day. After Giemsa staining, the number of parasite-infected RBCs among a total of 10,000 RBCs was counted microscopically for calculation of parasitemia (Pr) as Eq. (2):

$$\text{Pr (%) = Number of RBCs infected with parasites/10,000 \times 100}$$  \hspace{1cm} (2)

The inhibition rate (IR) at each time point and dosing group was calculated by Eq. (3):  

$$\text{IR}_{\text{treatment}} (\%) = (1 - \frac{\text{Pr}_{\text{treatment}}}{\text{Pr}_{\text{negative}}}) \times 100$$  \hspace{1cm} (3)

The clearance rate refers to the percent of mice with complete elimination of P. falciparum (Pr = 0) in their RBCs accounting for the total number of mice in each group, and the median
parasite clearance time (PC_{50}) of mice in each group was estimated.

2.14. Receptor occupancy (RO) assays

The RO rate on erythrocytes after in vitro incubation of meplazumab with human peripheral blood was measured using flow cytometry. Peripheral venous blood was collected from four volunteers, and a series of different concentrations of meplazumab was spiked in for in vitro incubation. The drug on the blood cell surface was then detected using a biotin labeled anti-human IgG antibody. Erythrocytes were gated using CD235a which is an RBC surface marker, and the RO rate was then calculated. The drug concentrations and RO rates were fitted to obtain a drug concentration–receptor occupancy level curve. This curve was then used to calculate the concentration of meplazumab added at a RO of 10%, 20%, 50%, and 90%.

2.15. Statistical analysis

The statistics of P and IR in antimalarial activity study of meplazumab are presented as the mean ± SEM. The statistics of exposure of the analytes (AUC and Cmax) were performed after being converted to logarithm scale. The same parameters in comparison between two groups used a t test, and the relationship between drug exposure and dose was statistically analyzed using SPSS Statistics 21 (IBM, Armonk, NY, USA).

3. Results

3.1. Meplazumab exhibits high affinity with CD147 from humans and cynomolgus monkeys

To select relevant species for toxicology studies, the affinity of meplazumab with CD147 from different species was evaluated.

As shown in Fig. 1A and B, the affinity constants (K_D values) in humans, and cynomolgus monkeys were determined to be 1.51 \times 10^{-10} and 1.26 \times 10^{-9} mol/L, respectively. Meplazumab was confirmed to bind to CD147 humans and cynomolgus monkeys. The K_D value of human CD147 was higher than that in cynomolgus monkeys.

Furthermore, meplazumab binding to CD147 expressed on erythrocytes from different species was also evaluated using flow cytometry. Meplazumab was able to bind erythrocytes of humans and cynomolgus monkeys. Meplazumab exhibited higher level of binding with erythrocytes of humans than that with erythrocytes of cynomolgus monkeys (Fig. 1C and Supporting Information Table S1).

Taken together, these results indicated that meplazumab exhibited binding activity to CD147 from humans and cynomolgus monkeys. The affinity in humans is 13.5-fold higher than that in cynomolgus monkeys. Compared with the amino acid sequences of CD147 in homo sapiens, the homology of CD147 in cynomolgus monkeys is 85%. The binding of meplazumab with the RBCs of cynomolgus monkeys also indicated that this species is appropriate for toxicology evaluation of meplazumab.

3.2. ADCC activity of meplazumab

The life cycle of P. falciparum in erythrocytic stage occurs in human erythrocytes. Previous studies have demonstrated the treatment of various diseases depends on ADCC activity of antibody.

To evaluate ADCC activity of meplazumab on human cells, an ADCC assay in vitro was conducted in this study. As shown in Fig. 1D and Supporting Information Table S2, meplazumab showed weak cytotoxicity (<7.51%) to A549 cells overexpressing CD147, but no clear concentration-dependent relationship was found among the groups and the concentration for 50% of maximal effect could not be calculated. Meplazumab could not induce ADCC activity at concentrations up to 1 mg/mL. In contrast, metuzumab, the positive control, showed significant cytotoxicity among the dose groups. We inferred that meplazumab had no ADCC effect on human erythrocytes.

3.3. Hemolysis studies in New Zealand white rabbits and cynomolgus monkeys

To further evaluate the effect of meplazumab on erythrocytes, hemolysis studies in vitro were conducted. The whole blood cells were collected from New Zealand white rabbits and cynomolgus monkeys. As shown in Supporting Information Tables S3 and S4, no hemolysis and RBC aggregation were observed for the test article, negative control or vehicle control at 15, 30, 45, 60, 120 and 180 min after incubation. The positive control tube was red and transparent at the above time points. Above all, meplazumab at 2 mg/mL did not cause hemolysis or aggregation of RBCs isolated from New Zealand white rabbits and cynomolgus monkeys.

3.4. Repeat-dose toxicity profiles of meplazumab in cynomolgus monkeys

In the repeat-dose toxicity study, one female animal in the 12 mg/kg group died on Day 22 due to excessive anesthesia. All other animals survived until the scheduled necropsy. No meplazumab-related changes were observed in clinical observations, body weight, food consumption, rectal temperature, ophthalmology, coagulation, clinical chemistry, immune function, bone marrow smear, urinalysis, or occult blood in feces examination in all animals during the whole study (Supporting Information Figs. S1 and S2, and Tables S5–S13).

For clinical hematology, compared with the 0 mg/kg group, increased percentage reticulocyte count was observed in males and females in the 6 and 12 mg/kg groups at the end of the dosing period (Supporting Information Tables S7 and S8). These changes were completely reversed at the end of the recovery period.

For clinical chemistry, as shown in Supporting Information Tables S9 and S10, in the 2 mg/kg group, no significant changes were observed in either males or females in comparison with the 0 mg/kg group. However, increased alanine aminotransferase in 1 of 5 females were observed on Day 28, as compared with the base values prior to administration. In the 6 mg/kg group, no significant changes were observed in either males or females in comparison with the 0 mg/kg group. However, increased alanine aminotransferase in 1 of 5 females and increased total bile acid in 1 of 5 females were observed on Day 28, as compared with the base values prior to administration.

As compared to the 0 mg/kg group, sporadic and dose-independent significant changes were considered unrelated to meplazumab and toxicologically insignificant. There were no meplazumab-related macroscopic or microscopic findings noted at
any of the dose levels. Under the experimental conditions of this study, the major changes of meplazumab on cynomolgus monkeys included increased percentage reticulocyte count dosed at >6 mg/kg, considered to be a pharmacodynamics effect. The above alterations were reversible. Under the conditions of this study, no observed adverse effect level was considered to be at 12 mg/kg with corresponding AUC$_{0-168}$ h of 19,123 µg·h/mL in males and 11,830 µg·h/mL in females.

Figure 1  *In vitro* characterization of meplazumab. The kinetic analysis of meplazumab binding to (A) human, and (B) cynomolgus monkey CD147 was performed by surface plasmon resonance measurements using a ProteOn XPR 36. (C) Meplazumab binding to red blood cells from different species was detected by flow cytometry. Meplazumab affinity was determined using median fluorescence intensity (MFI). (D) ADCC activity of meplazumab *in vitro*. The cytotoxicity of meplazumab and metuzumab on human peripheral blood mononuclear cells was measured using lactate dehydrogenase release assay. A549 cells expressing CD147 were used as target cells. Hu, human; Cyno, cynomolgus monkey.
3.5. Repeat-dose toxicokinetics studies of meplazumab in cynomolgus monkeys

The \textit{in vivo} repeated-dose toxicokinetics study in cynomolgus monkeys was performed together with repeated-dose toxicity study in cynomolgus monkeys. The results are shown in Fig. 2A–D and Table 1. For the animals dosed with meplazumab in the range of 2–12 mg/kg, the serum concentration of meplazumab increased with the dose level during the first and fourth dosing. There was no statistical difference of \( \text{AUC}_{0−168 \text{h}} \) and \( C_{\text{max}} \) between male and female animals during the first and fourth dosing in each group. As the dose increased from 2 to 12 mg/kg, the \( C_{\text{max}} \) increased in a greater than dose-dependent manner. For the animals dosed with the meplazumab in the range of 2–12 mg/kg, \( C_{\text{max}} \) and \( \text{AUC}_{0−168 \text{h}} \) did not have a proportionality with dose levels during the first and fourth dosing.

As shown in Table 1, after 4 weeks of repeated intravenous administration, no significant increase in exposure (\( \text{AUC}_{0−168 \text{h}} \)) or accumulation of drug exposure was observed in low-, medium- and high-dose groups of meplazumab, and no accumulated exposure was observed in both females and males. The \( \text{AUC}_{0−168 \text{h}} \) ratio (fourth dosing \textit{versus} first dosing) in female

![Figure 2](image)

\textbf{Figure 2} Serum concentration profiles of meplazumab in the cynomolgus monkeys. The mean serum concentration–time course of meplazumab after the first intravenous dose in (A) female animals and (B) male animals. The mean serum concentration–time course of meplazumab after the fourth intravenous dose in (C) female animals and (D) male animals. (E) The ratio of the individual \( C_{\text{max}} \) of the fourth to first injection of meplazumab in ADA-positive and ADA-negative cynomolgus monkeys in the different repeat-dose groups during the dosing period. (F) The ratio of individual \( \text{AUC}_{0−168 \text{h}} \) of the fourth to first injection of meplazumab in ADA-positive and ADA-negative cynomolgus monkeys in the different repeat-dose groups during the dosing period.
animals were 0.60, 1.27 and 1.02, respectively in low-, medium- and high-dose groups of meplazumab. The $AUC_{0–168\ h}$ ratio (fourth dosing versus first dosing) in male animals were 0.61, 1.50 and 1.97 respectively in low-, medium- and high-dose groups of meplazumab.

### 3.6 Effect of ADA on drug exposure in cynomolgus monkeys

The immunogenicity study was performed together with the repeated-dose toxicity study in cynomolgus monkeys. ADA was detected in 8/10 animals in 2 mg/kg group, 6/10 animals in 6 mg/kg group and 4/10 animals in 12 mg/kg group at the end of the recovery period (Table 2). Among the 187 tested samples, 58 were confirmed as positive, making the positive rate of 31.02%. The results of screening and confirmation tests indicated that positive animals were detected in all groups. ADA was detected more frequently in the 2 mg/kg group than in higher dose groups.

In 6 mg/kg group, the ratios of $C_{\text{max}}$ of the fourth to first injection of meplazumab in ADA positive animals were similar to that in ADA negative animals, while in 2 mg/kg group and 12 mg/kg group, higher variability of the ratios of $C_{\text{max}}$ was exhibited between ADA positive animals and ADA negative animals (Fig. 2E). The ratio of $AUC_{0–168\ h}$ of the fourth to first injection of meplazumab in ADA positive animals were lower than that in ADA negative animals (Fig. 2F). As shown in Table 3, The $AUC_{0–168\ h}$ of ADA positive animals in low, medium and high dose levels, respectively, lower than that of ADA negative animals in low, medium and high dose levels, respectively. The variability of $C_{\text{max}}$ and $AUC_{0–168\ h}$ in ADA positive animals was higher than that in ADA negative animals. It is indicated that the presence of ADA in ADA-positive animals was associated with accelerated drug clearance.

### 3.7 Tissue cross-reactivity study

To further evaluate the safety of meplazumab, a tissue cross-reactivity study was evaluated in FFPE slices from 32 kinds of normal human tissues and 33 kinds from normal tissues of cynomolgus monkeys. Negative staining with meplazumab at the low concentration (1 μg/mL) was observed in all kinds of normal human and cynomolgus monkey tissues. At the high concentration (20 μg/mL) of meplazumab, negative staining was found in 25 kinds of normal human tissues and 33 kinds of normal cynomolgus monkey tissues, and weak to moderate staining was observed in 7 kinds of human tissues, including the stomach (3/4 in parietal cells), kidney (1/3 in renal tubular epithelial cells), heart (1/3 in myocardial cells), liver (4/8 in hepatic cells), bladder (2/3 in urothelial cells), adrenal gland (2/3 in adrenal cortical epithelial cells) and prostate (2/3 in prostatic epithelial cells). In addition, CD31-positive staining was found in endothelial cells of all tissues (except for human spinal cord slices and human bone marrow smears, in which no endothelial cells were observed), indicating that the antigen was well preserved in all tissues; moreover, negative staining was found in all tissues in PBS blank control staining. In positive tissue control staining, positive membrane and cytoplasm staining in both human lung adenosquamous carcinoma tissues and human hepatocellular carcinoma tissues were observed after staining with meplazumab at low (1 μg/mL) and high (20 μg/mL) concentrations. Based on the above results, meplazumab had a low cross-reactivity rate in various tissues.

### 3.8 Distribution and excretion in SD rats

The individual tissue distribution of the TCA-precipitated peptide of $^{125}$I-mepazumab in the rats is summarized in Fig. 3A. The

### Table 1 Toxicokinetic parameters of meplazumab in cynomolgus monkeys in repeat-dose toxicity study.

| Group  | Gender | Sample | $C_{\text{max}}$ (μg/mL) | $AUC_{0–168\ h}$ (μg·h/mL) | $T_{\text{max}}$ (h) |
|--------|--------|--------|--------------------------|---------------------------|------------------|
|        |        |        | Median | Min | Max | Median | Min | Max |
| 2 mg/kg | Male | Day 1  | 25 ± 9 | 853 ± 156 | 0.25 | 0.25 | 24.00 |
|        |       | Day 22 | 28 ± 11 | 508 ± 509 | 0.25 | 0.25 | 0.25 |
|        | Female | Day 1 | 30 ± 4 | 1010 ± 71 | 0.25 | 0.25 | 4.00 |
|        |       | Day 22 | 28 ± 18 | 640 ± 808 | 0.25 | 0.25 | 0.25 |
| 6 mg/kg | Male | Day 1 | 117 ± 5 | 3755 ± 607 | 0.25 | 0.25 | 0.25 |
|        |       | Day 22 | 155 ± 29 | 5287 ± 2092 | 0.25 | 0.25 | 0.25 |
|        | Female | Day 1 | 112 ± 30 | 4513 ± 843 | 0.25 | 0.25 | 0.25 |
|        |       | Day 22 | 158 ± 22 | 5551 ± 1208 | 0.25 | 0.25 | 0.25 |
| 12 mg/kg | Male | Day 1 | 234 ± 22 | 9821 ± 1475 | 0.25 | 0.25 | 0.25 |
|        |       | Day 22 | 307 ± 32 | 19,123 ± 2726 | 0.25 | 0.25 | 0.25 |
|        | Female | Day 1 | 247 ± 20 | 11,375 ± 1403 | 0.25 | 0.25 | 0.25 |
|        |       | Day 22 | 256 ± 136 | 11,830 ± 10,649 | 0.25 | 0.25 | 0.25 |

Day 1, first dosing; Day 22, fourth dosing; the $C_{\text{max}}$ and AUC results represent the mean ± SEM.

### Table 2 Immunogenicity detection results in cynomolgus monkeys in repeat-dose toxicity study.

| Dosing (mg/kg) | Number of animals (M/F) | Number of positive animals | Proportion of positive animals (%) |
|---------------|-------------------------|----------------------------|-----------------------------------|
| 0             | 5/5                     | 0                          | 0                                 |
| 2             | 5/5                     | 8                          | 80                                |
| 6             | 5/5                     | 6                          | 60                                |
| 12            | 5/5                     | 4                          | 40                                |

M, male; F, female. The results were collected from the immunogenicity detection of all surviving animals before necropsy during the dosing and recovery periods.
radioactive precipitation exposure $\text{AUC}_{0 \rightarrow 240} \text{ h}$ was highest in the plasma and lowest in the brain. Using radioactivity of precipitation to calculate the AUC, the AUC in each tissue was listed in the following order: plasma $>$ whole blood $>$ ovary $>$ lung $>$ blood cells $>$ adrenal gland $>$ liver $>$ kidney $>$ heart $>$ mesentery lymph nodes $>$ fat $>$ spleen $>$ femur $>$ testis $>$ intestine $>$ thymus $>$ stomach $>$ urine $>$ skeletal muscle $>$ brain. As shown in Figs. 3B, $^{125}\text{I}$-meplazumab underwent a rapid and widespread distribution in tissues throughout the whole body during the experiment. In most tissues and organs, the radiation concentration of $^{125}\text{I}$-meplazumab reached its maximum distribution of 2 h after administration, and gradually decreased over time. At 240 h after administration, the radiation concentration of $^{125}\text{I}$-meplazumab was mainly distributed in plasma, whole blood and blood cells (Fig. 3B). At each time point, the radiation concentration of $^{125}\text{I}$-meplazumab in brain and skeletal muscle was very low, suggesting that meplazumab did not pass through the blood–brain barrier and did not accumulate in skeletal muscle tissue (Fig. 3B). The gamma counting results of different fractions of plasma and urine after HPLC separation showed that the corresponding peak of $^{125}\text{I}$-meplazumab appeared at 10.3 min. No radioactive peak was observed from the urine at all-time points, indicating that the drug was mainly present as the form of metabolites in urine (HPLC data not provided). The total radiation content in the thyroid at 2 and 240 h after administration accounted for $0.08 \pm 0.03\%$ and $0.04 \pm 0.02\%$ of the radioactivity dose, respectively, indicating that $^{125}\text{I}$-meplazumab did not accumulate in thyroid tissue.

### Table 3

| Dose (mg/kg) | ADA   | $n$ | $C_{\text{max}}$ (µg/mL) Mean ± SEM | CV% | AUC$_{0 \rightarrow 168}$ h (µg·h/mL) Mean ± SEM | CV% |
|--------------|-------|----|-----------------------------------|-----|-----------------------------------------------|-----|
| 2            | Negative | 4  | 41.9 ± 9                         | 22  | 1255 ± 391                                   | 31  |
|              | Positive | 6  | 19.3 ± 8                         | 42  | 120 ± 162                                    | 135 |
| 6            | Negative | 7  | 155.3 ± 29                      | 19  | 6230 ± 847                                   | 14  |
|              | Positive | 3  | 150.4 ± 7                        | 4   | 3526 ± 1389                                  | 39  |
| 12           | Negative | 7  | 312.3 ± 44                       | 14  | 19,768 ± 2626                                 | 13  |
|              | Positive | 3  | 209.4 ± 160                     | 76  | 54,644 ± 8523                                 | 156 |

ADA, anti-drug antibody; $n$, number of animal; the data of $C_{\text{max}}$ and AUC$_{0 \rightarrow 168}$ h was collected from the individuals during the first and fourth injection of meplazumab.
A time course of the excreted radioactivity derived from $^{125}$I-meplazumab in urine, feces, and bile is shown in Fig. 3C and D. Forty-eight hours after injection of $^{125}$I-meplazumab, the excretion rates of radioactivity into the bile, urine, and feces were $3.1 \pm 1.1\%$, $14.1 \pm 1.0\%$, and $1.2 \pm 0.3\%$, respectively. At 1176 h, the excretion rates in the urine and feces were $68.7 \pm 8.0\%$ and $17.2 \pm 4.0\%$, respectively, and the total excretion rate in the urine and feces was $85.9 \pm 7.7\%$.

Based on these results, the radiolabeled drugs were mainly excreted by the urine and a small portion was eliminated in the feces.

3.9. Activity of meplazumab against chloroquine-resistant strain Dd2 in huRBC-engrafted NOG mice

Zhang et al. confirmed the antimalarial efficacy of meplazumab on chloroquine-sensitive and -resistant P. falciparum in vitro. To further confirm that meplazumab also has a good therapeutic effect on drug-resistant strains of P. falciparum in vivo, we established a human erythrocyte chimeric NOG mouse model infected with P. falciparum Dd2 and evaluated the antimalarial efficacy of meplazumab in the constructed humanized mice. NOG mice were routinely observed after administration.

The results showed that compared with the human IgG group, parasitemia in the peripheral blood of mice treated with meplazumab ($P = 0.0031$) was significantly reduced (Fig. 4A and Supporting Information Table S15); compared with the chloroquine group, parasitemia in the peripheral blood of mice treated with meplazumab ($P = 0.0002$) was significantly reduced (Fig. 4A and Supporting Information Table S15). The chloroquine group had no significant inhibition after administration, while there was a significant difference between meplazumab and chloroquine on the parasite inhibition rate ($P < 0.0001$, Fig. 4B).

As shown in Supporting Information Table S16 and Fig 4C, 5 mg/kg meplazumab completely cleared malaria parasites from peripheral blood in humanized mice on Day 5, while chloroquine did not. The median parasite clearance time in the meplazumab group was significantly shorter than that of the human IgG group, with a PC50 value of 2.5 days ($P < 0.0001$). The PC50 of chloroquine was 7 days, and no significant difference was observed between chloroquine and human IgG ($P = 0.5188$).

3.10. RO assays

To determine the relationship between drug concentration and RO rate on erythrocytes after incubation of meplazumab with human peripheral blood and to provide reference for the initial dose in a clinical trial, RO% on erythrocytes after in vitro incubation of meplazumab with human peripheral blood was measured using flow cytometry. As shown in Fig. 5, the drug concentrations and the RO% were plotted to obtain a drug concentration–receptor occupancy level curve. The concentration of meplazumab at different RO rates was calculated from the model. Under the assumption of exclusively intravascular distribution and a blood volume of a healthy adult of approximately 77 mL/kg, meplazumab doses were projected. Maximum concentrations ($C_{\text{max}}$) required for a 10%, 20%, 50%, and 90% RO were determined and the corresponding projected doses were $0.511 \pm 0.080$, $0.820 \pm 0.11$, $1.634 \pm 0.21$ and $4.169 \pm 0.71$ µg/mL, respectively. When meplazumab exhibited intravascular distribution, the doses at the RO levels of 10%, 20%, 50% and 90% of $C_{\text{max}}$ were predicted to be 39.3, 63.2, 125.8, and 321.0 µg/kg, respectively.

4. Discussion

In recent years, increasing number of studies have shown that CD147 plays an important role in the invasion of erythrocytes by P. falciparum. CD147 and its ligand proteins are becoming potential targets for antimalarial therapy. Meplazumab is a humanized IgG2 mAb targeting human CD147 and is indicated for inhibitory prevention and treatment of P. falciparum infection. Repeat-dose toxicology studies of meplazumab have not been reported. To identify potential animal models for toxicology studies, we first determined the binding affinity of meplazumab to CD147 from different species. The results indicated that meplazumab could bind to CD147 from humans and cynomolgus monkeys. The affinity in humans is 13.5-fold higher than that in cynomolgus monkeys (Fig. 1A and B and Supporting Information Table S16).
needs to be further studied in clinical trials. The immunogenicity of meplazumab in the human body is affected by the species difference between humans and cynomolgus monkeys. Furthermore, because of the assay sensitivity, drug tolerance of the assay, drug administration route, time of sampling, and state of health. Therefore, the immunogenicity detection results demonstrated that meplazumab was well tolerated in the monkeys. No mortalities, significantly adverse reactions, or toxicities were observed in the treated animals. No changes in blood temperature, food consumption, or platelet counts were observed. No respiratory, cardiovascular, ophthalmological, or central nervous system impairments were noted in any of the treated animals. Increased percentage reticulocyte count dosed at >6 mg/kg was considered to be a pharmacodynamic effect, which should be used as an important indicator in clinical trials (Supporting Information Tables S7 and S8). No significant increase in exposure (AUC₀ to 168 h) or accumulation of drug exposure was observed after 4 weeks of repeated intravenous administration (Table 1). No observed adverse effect level was estimated to be 12 mg/kg in monkeys.

ADA was detected in all surviving cynomolgus monkeys after repeat intravenous administration of meplazumab, and it may accelerate drug clearance and reduce exposure to drugs (Fig. 2E and F, and Table 3). However, the immunogenicity detection results depend largely on different factors, such as the type of assay, assay sensitivity, drug tolerance of the assay, drug administration route, time of sampling, and state of health. Furthermore, because of the species difference between humans and cynomolgus monkeys, the immunogenicity of meplazumab in the human body needs to be further studied in clinical trials.

The results of a normal human tissue cross-reactivity study showed that there was no cross-reactivity of meplazumab towards the lung, spleen, colon, small intestine or ovary. The tissues with a cross-reactivity rate of ≤20% included the liver, brain and testis. The tissues with a cross-reactivity rate of >20% included the heart and kidney (myocardial cells and renal tubular epithelial cells). This indicates that meplazumab has a low tissue cross-reactivity and high safety, and suggests that we should pay attention to the effect of meplazumab on the function of the human heart, kidney, etc. in clinical studies.

To evaluate the therapeutic potential of meplazumab, a study on the distribution and excretion of meplazumab was conducted in SD rats. Our data showed that meplazumab was mainly distributed in plasma, whole blood and blood cells making it more effective and specific in to clearing parasites in the blood. In addition, meplazumab did not pass through the blood-brain barrier and did not accumulate in skeletal muscle tissue. Meplazumab was mainly excreted by the urine.

A previous research confirmed that meplazumab blocked the interaction between CD147 and the RAP2 ligand, and inhibited the formation of parasitophorous vacuoles, thus blocking the route of P. falciparum to invade huRBCs, which is a different mechanism from other anti-falciparum malaria drugs. Our results showed that meplazumab had no ADCC effect on human erythrocytes at concentrations up to 1 mg/mL. To some extent, it is confirmed that the antimalarial efficacy of meplazumab is to block the interaction between CD147 and RAP2 through Fab segment rather than ADCC activity of Fec segment. In addition, we established a chloroquine-resistant strain Dd2-infected humanized mouse model for the first time. Combined with previous studies, meplazumab at 5 mg/kg completely eliminated chloroquine-sensitive and -resistant parasites in humanized mice while chloroquine was ineffective against this resistant strain at 5 mg/kg, implicating that the efficacy of meplazumab is superior to that of chloroquine. Taken together, it is believed that meplazumab is an erythrocytic stage macromolecular antibody drug efficacious in the control of clinical occurrence and the inhibitory treatment of severe malaria.

5. Conclusions

In conclusion, the results from this study suggest that meplazumab is safe, tolerated, and effectively inhibits the chloroquine-resistant Dd2 strain of P. falciparum from invading huRBCs, and the efficacy was superior to that of chloroquine in vivo, which is promising as an efficacious drug for the cure of P. falciparum.

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Author contributions

Zhinan Chen, Ping Zhu, Huijie Bian, Zheng Zhang and Kun Zhang conceived and designed this study. Kun Zhang, Zheng Zhang, Yu Zhao performed experiments and analyzed the data. Mengyao Zhang and Xiaodong Wu performed partial experiments. Zhinan Chen, Ping Zhu and Huijie Bian drafted and revised the manuscript. The manuscript has been reviewed and approved by all authors.

Conflicts of interest

The authors have declared that no conflicts of interest exist.
Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.aspbs.2020.06.011.

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