In Vivo Antimalarial Activity of Nanoparticles Enhanced Artemether lumefantrine (Coatem) on Plasmodium berghei Infected Mice

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Abstract

The search for newer antimalarial agents in the light of drug resistance has become a labret to researchers with no breakthrough and with malaria research funds being cut down, is a race against time. In this research magnesium oxide nanoparticles (MgO NPs) were used to enhance the activity of coatem and administered to mice orally. 36 albino mice were used. Mice were infected with ANKA strain of Plasmodium berghei and were kept in the laboratory for three days before initial parasitamia were carried out to ascertain the level of parasitamia. MgO nanoparticles and coatem were given to the mice in graded doses. Nanoparticles alone was given to one group at 200mg/kg and coatem at 20mg/kg to another group as a positive control and a group was left without treatment as a negative control, the other three groups were given 10mg/kg,5mg/kg and 2.5mg/kg coatem and 70mg/kg MgO. result obtained after microscopy were, group 200mg/kg nanoparticles cleared all the parasite, group coatem 20mg/kg had 91.7% clearance, group no treatment had an increase in parasitamia from 21.8% to 40.8%, group 10mg/kg coatem and 70mg/kg nanoparticles had 98.6% clearance rate, group 5mg/kg coatem and 70mg/kg had 97.6% clearance rate and the last group 2.5mg/kg and 70mg/kg had 96.4% clearance rate. There was a statistically significant difference between groups as determined by one way ANOVAs F (3, 20) =6.16, P=0.003 and then Tukey HSD P=0.001. With this it was a clear indication that nanoparticles were more active than coatem and coatem had a better clearance rate when nanoparticles were added to it.

Keywords

Coatem; Nanoparticles; Plasmodium; Magnesium Oxide; ANKA Strain

Introduction

The search of a malaria vaccine had since proven to be a mirage and in its absence antimalarial drug remain the only method to control illness and mortality due to Plasmodium falciparum malaria. Although several anti-plasmodial drugs are available P. falciparum has gradually developed resistance to most if not all of them. The prevalence and resistance are increasing and the channels for creating new agents are drying out [1] with all the advance research in malaria and malaria drugs development and with the introduction of artemisinin combination therapy (ACTs) as a first choice of treating uncomplicated malaria, malaria still kills thousands of people around the globe.

Even after taking the proposed treatment relapse of fever occurs just a few days after and additional treatment is administered for a complete cure. If this is happening that means is either the magic drug no longer can be able to give total clearance of parasite in the three days as it is supposed to or the malaria parasite has develop a means of resisting the drug as shown by past studies so that a longer treatment time is required or the drug is not delivered to the target.
Development of either new antimalarial drugs using available compounds or new ways to carry the antimalarial agents to specific target cells is required for better treatment of the parasite in cases of infection. This may be very expensive and time consuming but the end result may be worth the try because the spread of antimalarial resistance around the world can occur very fast and more effort must be put into the war against it.

Early diagnosis and treatment of malaria reduces disease and prevents deaths. It also contributes to reducing transmission. WHO recommends that all suspected cases of malaria be confirmed using parasite based testing before administering treatment. Treatment only on the bases of symptoms alone should only be considered when parasitological diagnoses are not possible WHO [2].

In this research, magnesium oxide nanoparticles were synthesised and used as a potential new antimalarial agent or antimalarial enhancing agent. This was to check the anti-plasmodial activities of nanoparticles on antimalarial drugs and to check the efficacy of the drug on *Plasmodium* infected red blood cell *in-vivo*. The reason why Nanomaterials was a point of focus in this research is because they have been reported to possess unique, beneficial, chemical, physical and mechanical properties in cancer research and they can be used for a wide variety of application due to high sensitivity sensors, act as nanocatalyst and have longer lasting medical implants.

**Materials and Methods**

**Synthesis and Characterization of Magnesium Oxide Nanoparticles**

Magnesium oxide nanoparticles were synthesized according to the procedure of Rizwan et al. [3] using magnesium nitrate (MgNO$_3$·6H$_2$O) as a source material with sodium hydroxide, 8g of magnesium nitrate and 2.4g of polyethylene glycol was dissolved in 200 ml of deionized water. 1M (4g in 100ml distilled water) sodium hydroxide solution was added drop wise to the prepared deionised water. 1M (4g in 100ml distilled water) sodium hydroxide, 8g of magnesium nitrate and 2.4g of polyethylene glycol was dissolved in 200 ml of deionized water. 1M (4g in 100ml distilled water) sodium hydroxide solution was added drop wise to the prepared magnesium nitrate solution while stirring it continuously on a magnetic stirrer at 60°C. White precipitate of magnesium hydroxide appeared in a beaker after few minutes. The stirring was continued for 30 minutes. The pH of the solutions was increased to 11.5, as measured by the pH meter. The precipitate was filtered and washed with methanol five times to remove ionic impurities and then centrifuged for 5 minutes at 1500 rpm/min and dried at room temperature for a week. The dried white powder samples were annealed in an oven for twenty four hours at 200°C. The morphological investigation was carried out by the scanning electron microscopy Wagh et al. [4], (SEM, Inspect S50) at the National Geological Survey Agency, Kaduna. Samples composition of the synthesized magnesium oxide nanoparticles were analyzed by the Fourier transform infrared (FTIR) spectroscopy at the Department of Biochemistry, Bayero University Kano, in the range of 650-4000 nm and infrared spectroscopy was carried out at a range between 200 and 800nm.

**Preparation of Stock Concentration of Nanoparticles**

Stock for the dose of 10mg/kg using 0.010g of the powdered MgO nanoparticles with 0.01g of acacia powder were dissolved in 10ml of normal saline to prepare MgO nanoparticles as stated by Ojurongbe et al. [5]. This was given to the experimental mice through the course of the experiment. The preparation was stored in a refrigerator during the experiment until required.

**Preparation of Stock Concentration of Artemether Lumefantrine Standard Drug 20mg/120mg (Coatem)**

Artemether lumefantrine branded tablets was obtained from Lamco Pharmaceutical store in Kano state and taken to the laboratory for the experiment. One tablet was grounded in a mortar and dissolved in 10ml of normal saline. This is to prepare a stock concentration of and serially diluted to make the graded doses of 10mg/kg, 5mg/kg and 2.5 mg/kg this was given as a single dose regimen after every 24hours.

**Experimental Animals and Methodology for Oral Drug Administration**

For the curative model, 36 white albino mice (Westar stock) and 2 infected donor mice were obtained from the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria. The animals were fed on diet specially prepared from chick grower, cassava flour and maize bran and were given water throughout the study period. The experimental animals were divided into 6(A1-A6) and numbered 1-6, respectively. Animals’ weights ranged from 18g to 26g just before the commencement of the experiment. Experimental design for curative model was used this involves the use of 36 mice of 6 each in a group and six groups were made. The animals were inoculated with the parasite by removing parasitological diagnoses are not possible WHO [2].
blood from an ocular puncture and about 10 drops of blood was collected in an EDTA container and 2 ml of normal saline was added and mixed well. 0.2 ml of this blood containing 1x10^7 of *Plasmodium berghei* parasitized red cells was injected into each mouse intraperitoneally. Mice were allowed rest for 3 days this is to enable the parasite time to multiply to the between 20-30% parasitamia mark. on the 3rd day, thin blood film were made from each mouse taken from the tail vein and stained using 3% geimsa for 45minutes this is to know the initial percentage of parasite in each mice before the start of treatment. Group A one were inoculated with no treatment, group A2 were given 200 mg/Kg of MgO NPs group A3 was given 20 mg/Kg coatem alone based on the weight of the mice drugs were administered, group A4 was given 10 mg/Kg coatem and 70 mg/Kg nanoparticles, group A5 was given 5 mg/Kg coatem and 70 mg/Kg nanoparticles, group A6 was given 2.5 mg/Kg coatem and 70 mg/Kg. Thin blood films were made from the tail of the mice after every 24 hours, stained using standard geimsa and read using a light microscope Optika-B 159 x100 oil emersion objective.

**Statistical Analysis**

One way analysis of variance (ANOVA) was used to compare the significant difference of the results and this was followed by the Tukey Honestly Significance Difference Test to determine where the difference lies.

**Results and Discussions**

**Scanning Electron Microscope Image of Synthesised Magnesium Oxide Nanoparticles**

The result clearly indicated that at lower doses of coatem combined with nanoparticles, there was better and more effective reaction on the parasite than when coatem alone was administered. Even the standard dose of 20 mg/Kg did not fully clear the parasite. This clearly shows that there is better clearance activity when MgO nanoparticles were used because at the lowest dose of coatem of 2.5 mg/Kg combine with 70 mg/Kg of nanoparticles the mean percentage clearance (99.4) rate is still better than the mean percentage clearance (98.5) when coatem was used alone. Statistical analysis showed that there was a significant difference between groups as determine by one way ANOVAs F (3, 20) =6.16, P=0.003 and then Tukey HSD P=0.001.

**Conclusion**

Even though coatem is the first line of treatment as recommended by the world health organization in malaria endemic regions, a lot of report has proof that it is no longer able to clear the parasite totally and relapse always occur and in the search for newer ones this research clearly

Figure 1 (A and B): Shows the MgO NPs were composed of sheets and plates which are closely stacked.
Figure 2: FTIR Analysis of Synthesised Magnesium Oxide Nanoparticles

Table 1: Mean Percentage Parasitamia at Day Three after Inoculation with \textit{P. berghei} Anka Strain

<table>
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<tr>
<th>S/N</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
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<td>24.2</td>
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<td>17.3</td>
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</table>

Table 1 shows the initial mean parasite load before the onset of treatment with Magnesium Oxide Nanoparticles Combined with Coatem

Table 2: Mean Percentage Clearance Rate of Coatem and Nanoparticles after Treatment

<table>
<thead>
<tr>
<th>A1(no treatment)</th>
<th>A2(200 mg/Kg MgO NPS)</th>
<th>A3(20 mg/Kg coatem)</th>
<th>A4(10 mg/Kg COATEM 70 mg/ml MgO)</th>
<th>A5(5 mg/Kg 70 mg/Kg MgO)</th>
<th>A6(2.5 mg/Kg 70 mg/Kg MgO)</th>
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<tbody>
<tr>
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Table 2 shows the result that was obtained when MgO nanoparticles were combined with coatem
indicates that other antimalarial agents are out there waiting to be uncovered. To the best of our knowledge this paper is the first that attempt to use MgO nanoparticles in-vivo on infected mice model. We show through our analysis that this research can be the breakthrough in the fight against malaria and antimalarial drug resistance by Plasmodium species.

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References


