ORIGINAL ARTICLE

# In vitro antiplasmodial activity of PDDS-coated metal oxide nanoparticles against *Plasmodium falciparum*

Samuel Jacob Inbaneson · Sundaram Ravikumar

**Abstract** Malaria is the most important parasitic disease, leading to annual death of about one million people and the *Plasmodium falciparum* develops resistant to well-established antimalarial drugs. The newest antiplasmodial drug from metal oxide nanoparticles helps in addressing this problem. Commercial nanoparticles such as $Fe_3O_4$, MgO, $ZrO_2$, $Al_2O_3$ and $CeO_2$ coated with PDDS and all the coated and non-coated nanoparticles were screened for antiplasmodial activity against *P. falciparum*. The $Al_2O_3$ nanoparticles ($71.42 \pm 0.49$ μg ml$^{-1}$) showed minimum level of $IC_{50}$ value and followed by MgO ($72.33 \pm 0.37$ μg ml$^{-1}$) and $Fe_3O_4$ nanoparticles ($77.23 \pm 0.42$ μg ml$^{-1}$). The PDDS-$Fe_3O_4$ showed minimum level of $IC_{50}$ value ($48.66 \pm 0.45$ μg ml$^{-1}$), followed by PDDS-MgO ($60.28 \pm 0.42$ μg ml$^{-1}$) and PDDS-$CeO_2$ ($67.06 \pm 0.61$ μg ml$^{-1}$). The PDDS-coated metal oxide nanoparticles showed superior antiplasmodial activity than the non-PDDS-coated metal oxide nanoparticles. Statistical analysis reveals that, significant in vitro antiplasmodial activity ($P < 0.05$) was observed between the concentrations and time of exposure. The chemical injury to erythrocytes showed no morphological changes in erythrocytes by the nanoparticles after 48 h of incubation. It is concluded from the present study that, the PDDS-$Fe_3O_4$ showed good antiplasmodial activity and it might be used for the development of antiplasmodial drugs.

**Keywords** Antiplasmodial activity · $IC_{50}$ · Metal oxide nanoparticles · *Plasmodium falciparum*

S. Jacob Inbaneson · S. Ravikumar (✉)
Department of Oceanography and Coastal Area Studies,
School of Marine Sciences, Alagappa University,
Thondi Campus, Ramanathapuram District,
Thondi 623 409, Tamil Nadu, India
e-mail: ravibiotech201321@gmail.com

## Introduction

Malaria caused by parasites of the genus *Plasmodium*, is one of the leading public health problems in Sub-Saharan Africa. It is estimated that malaria kills over a million annually and some 3.2 billion people living in 107 countries or territories are at risk (WHO 2005). In Sub-Saharan regions, 45 countries were endemic for malaria in 2008 (WHO 2008). Artemisinin combination treatments for falciparum malaria are currently the only first-line antimalarial drugs amenable to widespread use against all chloroquine-resistant malaria parasites (Barnes and Folb 2003). However, artemisinin-resistant malaria parasites were recently detected in Cambodia (Maude et al. 2009). The newest antiplasmodial drugs from biological sources help in addressing this problem (Ravikumar et al. 2011a, b, c, d, e) but it seems to be the loss of biodiversity and cost effective. The nanoparticles posses variety of biological activities (Martinez-Castanon et al. 2008; Lee et al. 2010; Singh et al. 2008) and considered as a rich source of novel antiplasmodial agents and these potential resources were scarcely explored. In the present study, we report the findings of antiplasmodial potential of metal oxide nanoparticles against *Plasmodium falciparum*.

## Materials and methods

### Nanoparticles

Commercial nanoparticles of $Al_2O_3$, $Fe_3O_4$, $CeO_2$, $ZrO_2$, and MgO were procured from Sigma Aldrich Company, India. The characteristics of the nanoparticles are represented in Table 1.

Springer

**Table 1** Characteristics of nanoparticles used for antiplasmodial assay

| Formula | Product number | Molecular weight | Form | Particle size (nm) |
|---------|----------------|------------------|------|--------------------|
| $Al_2O_3$ | 642991 | 101.96 | Dispersion | <50 |
| $CeO_2$ | 643009 | 172.11 | Dispersion | <25 |
| $Fe_3O_4$ | 700312 | 231.53 | Solution | 9–11 |
| $ZrO_2$ | 643025 | 123.22 | Powder | <100 |
| MgO | 203718 | 40.30 | Powder | <30 |

## Parasite cultivation

The antiplasmodial activity of nanoparticles was assessed against *P. falciparum* obtained from the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India. *P. falciparum* are cultivated in human $O Rh^+$ red blood cells using RPMI 1640 medium (HiMedia Laboratories Private Limited, Mumbai, India) (Moore et al. 1967) supplemented with $O Rh^+$ serum (10 %), 5 % sodium bicarbonate (HiMedia Laboratories Private Limited, Mumbai, India) and 40 $\mu g ml^{-1}$ of gentamicin sulfate (HiMedia Laboratories Private Limited, Mumbai, India). Hematocrits were adjusted at 5 % and parasite cultures were used when they exhibited 2 % parasitaemia (Trager 1987).

## In vitro antiplasmodial assay

Different concentrations of filter sterilized nanoparticles (100, 50, 25, 12.5, 6.25 and 3.125 $\mu g ml^{-1}$) were incorporated into 96-well tissue culture plate containing 200 $\mu l$ of *P. falciparum* culture with fresh red blood cells diluted to 2 % hematocrit in triplicates. Negative control was maintained with fresh red blood cells and 2 % parasitized *P. falciparum* diluted to 2 % hematocrit, positive control was maintained with parasitized blood cells culture treated with chloroquine and artemether (Azas et al. 2001). Parasitaemia was evaluated after 48 h by Giemsa stain and the average percentage suppression of parasitaemia was calculated by the following formula: Average % suppression of parasitaemia = Average % parasitaemia in control − Average % parasitaemia in test/Average % parasitaemia in control × 100.

## Antiplasmodial activity calculation and analysis

The antiplasmodial activities of nanoparticles were expressed by the inhibitory concentrations ($IC_{50}$) of the drug that induced a 50 % reduction in parasitaemia compared to the control (100 % parasitaemia). The $IC_{50}$ values were calculated (concentration of extract in $X$ axis and percentage of inhibition in $Y$ axis), using Office XP (SDAS)

software with linear regression equation. This activity was analyzed in accordance with the norms of antiplasmodial activity of Rasoanaivo et al. (1992). According to this norms, a nanoparticle is very active if $IC_{50} < 5$ $\mu g ml^{-1}$, active $5$ $\mu g ml^{-1} < IC_{50} < 50$ $\mu g ml^{-1}$, weakly active $50$ $\mu g ml^{-1} < IC_{50} < 100$ $\mu g ml^{-1}$ and inactive $IC_{50} > 100$ $\mu g ml^{-1}$.

## Chemical injury to erythrocytes

To assess any chemical injury to erythrocytes that might be attributed to the nanoparticles, 200 $\mu l$ of erythrocytes were incubated with 100 $\mu g ml^{-1}$ of the nanoparticles at a dose equal to the highest used in the antiplasmodial assay. The conditions of the experiment were maintained as in the case of antiplasmodial assay. After 48 h of incubation, thin blood smears were stained with Giemsa stain and observed for morphological changes under high-power light microscopy. The morphological findings were compared with those in erythrocytes that were uninfected and not exposed to nanoparticles (Waako et al. 2007).

## Results

A total of 11 samples were screened for antiplasmodial activity and the $IC_{50}$ values were represented in Table 2. Among the metal oxide nanoparticles, $Al_2O_3$ (71.42 ± 0.49 $\mu g ml^{-1}$) showed minimum level of $IC_{50}$ value and followed by MgO (72.33 ± 0.37 $\mu g ml^{-1}$) and $Fe_3O_4$ (77.23 ± 0.42 $\mu g ml^{-1}$). However, the PDDS-coated $Fe_3O_4$ showed minimum level of $IC_{50}$ value (48.66 ± 0.45 $\mu g ml^{-1}$)

**Table 2** Antiplasmodial ($IC_{50}$) activity of synthesised nanoparticles

| Sample | $IC_{50}$ ($\mu g ml^{-1}$) |
|--------|------------------------------|
| $Al_2O_3$ | 71.42 ± 0.49 |
| $CeO_2$ | 82.08 ± 0.51 |
| $Fe_3O_4$ | 77.23 ± 0.42 |
| $ZrO_2$ | 81.11 ± 0.53 |
| MgO | 72.33 ± 0.37 |
| PDDS-coated $Al_2O_3$ | 69.97 ± 0.46 |
| PDDS-coated $CeO_2$ | 67.06 ± 0.61 |
| PDDS-coated $Fe_3O_4$ | 48.66 ± 0.45 |
| PDDS-coated $ZrO_2$ | 79.66 ± 0.49 |
| PDDS-coated MgO | 60.28 ± 0.42 |
| PDDS | 49.63 ± 0.38 |
| Positive control | |
| Chloroquine | 19.59 ± 0.29 |
| Artemether | 4.09 ± 0.15 |

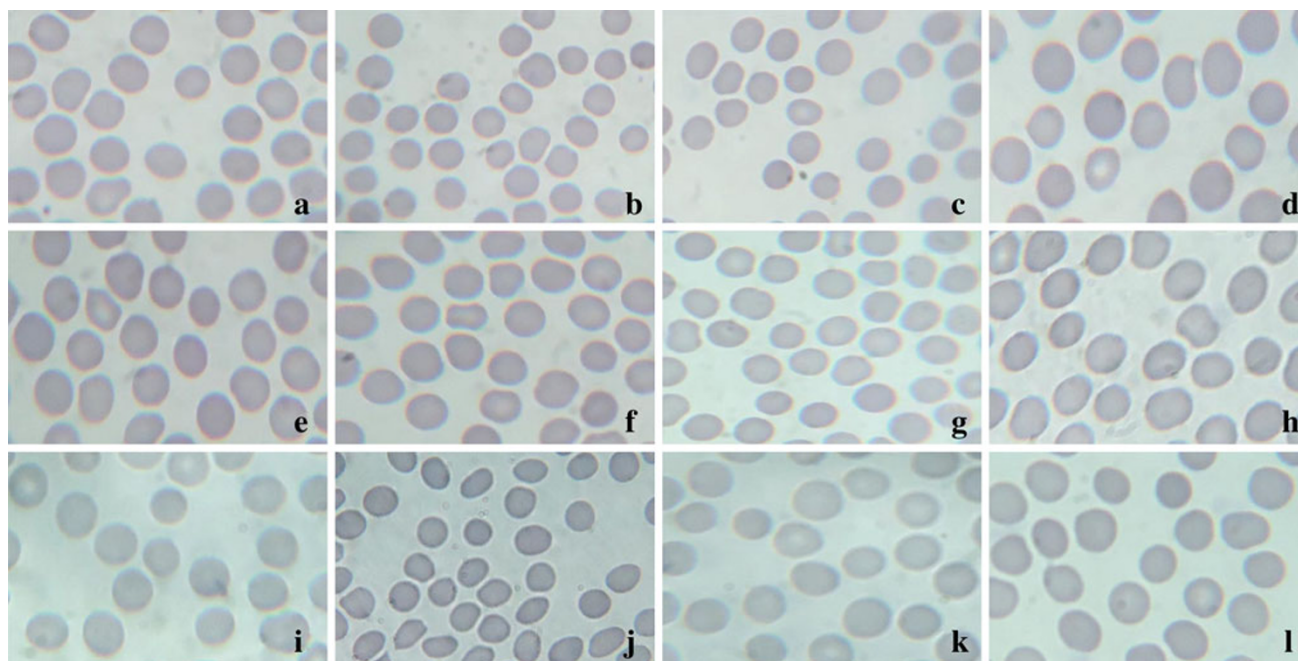Values are found significant between concentrations and time of exposure ($P < 0.05$)

**Fig. 1** Microscopical images of erythrocytes treated with synthesized nanoparticles. **a** $Al_2O_3$-treated erythrocytes; **b** $CeO_2$-treated erythrocytes; **c** $Fe_3O_4$-treated erythrocytes; **d** $ZrO_2$-treated erythrocytes; **e** MgO-treated erythrocytes; **f** PDDS-coated $Al_2O_3$-treated erythrocytes; **g** PDDS-coated $CeO_2$-treated erythrocytes; **h** PDDS coated $Fe_3O_4$-treated erythrocytes; **i** PDDS-coated $ZrO_2$-treated erythrocytes; **j** PDDS-coated MgO-treated erythrocytes; **k** PDDS-treated erythrocytes; **l** Uninfected and non-treated erythrocytes

and followed by PDDS-coated MgO ($60.28 \pm 0.42$ µg ml$^{-1}$) and $CeO_2$ ($67.06 \pm 0.61$ µg ml$^{-1}$). The PDDS alone showed $IC_{50}$ value of $49.63 \pm 0.38$ µg ml$^{-1}$ and the PDDS-coated metal oxide nanoparticles showed better antiplasmodial activity than the non-PDDS-coated metal oxide nanoparticles. The microscopic observation of uninfected erythrocytes added with all the test samples and uninfected erythrocytes from the blank column of the 96-well plate showed no morphological differences after 48 h of incubation (Fig. 1).

## Discussion

Metal nanoparticles have attracted much attention in the fields of physics, chemistry, electronics and biology (Lanje et al. 2010a; Schmid 1994; Henglein 1989) because of their unique electrical (Peto et al. 2002), chemical (Kumar et al. 2003) and optical (Krolikowska et al. 2003) properties, which are strongly dependent on the sizes and shapes of metal nanomaterials (Creighton and Eadon 1991; Zhang et al. 2000; Liu et al. 1995; Lanje et al. 2010a, b). Metal nanoparticles have a high specific surface area and a high surface-to-volume ratio. Nanostructured noble metals are potentially used in catalysis, optoelectronics and micro-electronics. Metal nanoparticles are particularly interesting systems because of the ease with which they can be synthesized and modified chemically (Ying et al. 2005).

However, the emergence of strains of *P. falciparum* resistant to chloroquine and many other drugs in succession and antimicrobial nature of nanoparticles has stimulated us to identify new antiplasmodial agents from nanoparticles.

The present study has tested 10 nanoparticles samples and a PDDS alone for antiplasmodial activity. Among the 10 nanoparticles, the PDDS-coated $Fe_3O_4$ showed minimum level of $IC_{50}$ value ($48.66 \pm 0.45$ µg ml$^{-1}$) and the concentration is 2.5-fold higher than the positive control chloroquine ($19.59 \pm 0.29$ µg ml$^{-1}$). According to Rasoanaivo et al. (1992), 10 % of the nanoparticles used by the present study are classified as active and 90 % of the samples are classified as weakly active (Fig. 2). The intracellular malarial parasite penetrates into the host red blood cell membrane through new permeation pathways (Go et al. 2004). It is already reported that, macromolecules like dextrans, protein A and IgG2a antibody also gain access to react with the parasite through the new permeation pathways (Pouvelle 1991). Foger et al. (2006) reported that, the antisense nanoparticles inhibited the malarial topoisomerase II in *P. falciparum*. The antisense oligodeoxynucleotides inhibit the growth of parasites in a non-specific manner by the polyanionic properties of oligonucleotides which interfere with the merozoite invasion into red blood cells (Noonpakdee et al. 2003; Barker et al. 1998). Moreover, the metal nanoparticles exhibited many antimicrobial activities (Kvitek et al. 2008; Marini et al. 2007; Holt and Bard 2005). Smaller size nanoparticles
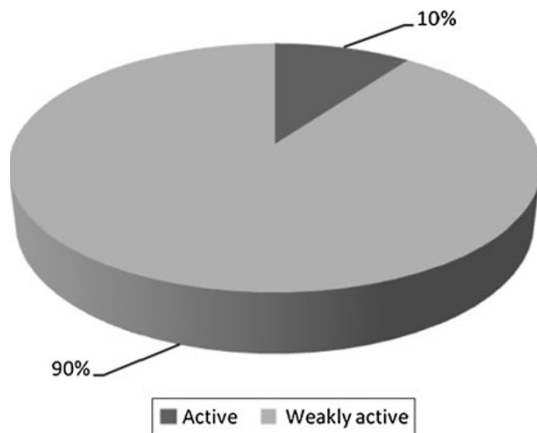
**Fig. 2** Percentage of antiplasmodial activity $IC_{50}$ values of metal oxide nanoparticles against *Plasmodium falciparum*

having the large surface area available for interaction and it might be a one of the reason for better antiplasmodial activity of PDDS-coated $Fe_3O_4$. It is concluded from the present study that, the PDDS-coated $Fe_3O_4$ nanoparticles could be used as an effective antiplasmodial agent for the management of malaria after successful completion of in vivo and clinical studies.

# References

Azas N, Laurencin N, Delmas F, Di Giorgio C, Gasquet M, Laget M, Timon David P (2001) Synergistic in vitro antimalarial activity of plant extracts used as traditional herbal remedies in Mali. Parasitol Res 88(2):165–171

Barker RH, Metelev V, Coakley A, Zamecnik P (1998) *Plasmodium falciparum*: effect of chemical structure on efficacy and specificity of antisense oligonucleotides against malaria in vitro. Exp Parasit 88:51–59

Barnes K, Folb P (2003) In reducing malaria's burden: evidence of effectiveness for decision makers; technical report. Global Health Council, Washington, pp 25–32

Creighton JA, Eadon DG (1991) Ultraviolet-visible absorption spectra of the colloidal metallic elements. J Chem Soc Faraday Trans 87(24):3881

Foger F, Noonpakdee W, Loretz B, Joojuntr S, Salvenmoser W, Thaler M, Bernkop-Schnurch A (2006) Inhibition of malarial topoisomerase II in *Plasmodium falciparum* by antisense nanoparticles. Int J Pharms 319:139–146

Go ML, Liu M, Wilairat P, Rosenthal J, Saliba KJ, Kirk K (2004) Antiplasmodial chalcones inhibit sorbitol-induced hemolysis of *Plasmodium falciparum*-infected erythrocytes. Antimicrob Agents Chemother 48:3241–3245

Henglein A (1989) Small-particle research: physicochemical properties of extremely small colloidal metal and semiconductor particles. Chem Rev 89(8):1861–1873

Holt KB, Bard AJ (2005) Interaction of silver ions with the respiratory chain of *Escherichia coli*: an electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar $Ag^+$. Biochem 44:13214–13223

Krolikowska A, Kudelski A, Michota A, Bukowska J (2003) SERS studies on the structure of thioglycolic acid monolayers on silver and gold. Surf Sci 532:227–232

Kumar A, Mandal S, Selvakannan PR, Parischa R, Mandale AB, Sastry M (2003) Investigation into the interaction between surface-bound alkylamines and gold nanoparticles. Langmuir 19:6277–6282

Kvitek L, Panacek A, Soukupova J, Kolar M, Vecerova R, Prucek R, Holecova M, Zboril R (2008) Effect of surfactants and polymers on stability and antibacterial activity of silver nanoparticles (NPs). J Phys Chem C 112:5825–5834

Lanje AS, Ningthoujam RS, Sharma SJ, Pode RB, Vatsa RK (2010a) Luminescence properties of $Sn_{1-x}Fe_xO_2$ nanoparticles. Int J Nanotech 7(9–12):979–988

Lanje AS, Sharma SJ, Pode RB (2010b) Magnetic and electrical properties of nickel nanoparticles prepared by hydrazine reduction method. Arch Phys Res 1(1):49–56

Lee SM, Song KC, Lee BS (2010) Antibacterial activity of silver nanoparticles prepared by a chemical reduction method. Korean J Chem Eng 27(2):688–692

Liu K, Nagodawithana K, Searson PC, Chien CL (1995) Perpendicular giant magnetoresistance of multilayered Co/Cu nanowires. Phys Rev B 51:7381

Marini M, De Niederhausern N, Iseppi R, Bondi M, Sabia C, Toselli M, Pilati F (2007) Antibacterial activity of plastics coated with silver-doped organic-inorganic hybrid coatings prepared by sol-gel processes. Biomacromolecules 8:1246–1254

Martinez-Castanon GA, Nino-Martinez N, Martinez-Gutierrez F, Martinez-Mendoza JR, Ruiz F (2008) Synthesis and antibacterial activity of silver nanoparticles with different sizes. J Nanopart Res 10:1343–1348

Maude RJ, Pontavornpinyo W, Saralamba S, Aguas R, Yeung S, Dondorp A, Day NPJ, White NJ, White LJ (2009) The last man standing is the most resistant: eliminating artemisinin-resistant malaria in Cambodia. Malaria J 8:31–131

Moore GE, Gerner RE, Frankin HA (1967) Cultures of normal human leukocytes. J Am Med Assoc 199:519–524

Noonpakdee W, Pothikasikorn J, Nimitsantiwog W, Wilairat P (2003) Inhibition of *Plasmodium falciparum* proliferation in vitro by antisense oligodeoxynucleotides against malarial topoisomerase II. Biochem Biophys Res Commun 302:659–664

Peto G, Molnar GL, Paszti Z, Geszti O, Beck A, Guczi L (2002) Electronic structure of gold nanoparticles deposited on SiOx/Si. Mater Sci Eng C 19:95–99

Pouvelle B (1991) Direct access to serum macromolecules by intraerythrocytic malaria parasites. Nature 353:73–75

Rasoanaivo P, Ratsimamanga Urverg S, Ramanitrhasimbola D, Rafatro H, Rakoto Ratsimamanga A (1992) Criblage d'extraits de plantes de Madagascar pour recherche d'activite antipaludique et d'effet potentialisateur de la chloroquine. J Ethnopharmacol 64:117–126

Ravikumar S, Jacob Inbaneson S, Suganthi P (2011a) Seaweeds as a source of lead compounds for the development of new antiplasmodial drugs from South East coast of India. Parasitol Res 109:47–52

Ravikumar S, Jacob Inbaneson S, Suganthi P, Gnanadesigan M (2011b) In vitro antiplasmodial activity of ethanolic extracts of mangrove plants from South East coast of India against chloroquine-sensitive *Plasmodium falciparum*. Parasitol Res 108:873–878

Ravikumar S, Jacob Inbaneson S, Suganthi P, Gokulakrishnan R, Venkatesan M (2011c) In vitro antiplasmodial activity of ethanolic extracts of seaweed macroalgae against *Plasmodium falciparum*. Parasitol Res 108:1411–1416

Ravikumar S, Jacob Inbaneson S, Suganthi P, Venkatesan M, Ramu A (2011d) Mangrove plants as a source of lead compounds for the development of new antiplasmodial drugs from South East coast of India. Parasitol Res 108:1405–1410

Ravikumar S, Ramanathan G, Jacob Inbaneson S, Ramu A (2011e) Antiplasmodial activity of two marine polyherbal preparations from *Chaetomorpha antennina* and *Aegiceras corniculatum* against *Plasmodium falciparum*. Parasitol Res 108:107–113

Schmid G (1994) Clusters and colloids: from theory to applications. VCH Verlagsgesellschaft, Weinheim

Singh M, Singh S, Prasada S, Gambhir IS (2008) Nanotechnology in medicine and antibacterial effect of silver nanoparticles. Digest J Nanomat Biostru 3(3):115–122

Trager W (1987) The cultivation of *Plasmodium falciparum*: applications in basic and applied research in malaria. Ann Trop Med Parasitol 82:511–529

Waako PJ, Katuura E, Smith P, Folb P (2007) East African medicinal plants as a source of lead compounds for the development of new antimalarial drugs. Afr J Ecol 45(1):102–106

WHO (2005) World Malaria Report. http://www.rbm.who.int/wmr2005/pdf/adve.pdf

WHO (2008) World Malaria Report (Visiting time: October 2009) http://www.who.int/malaria/wmr2008

Ying Z, Shengming J, Guanzhou Q, Min Y (2005) Preparation of ultrafine nickel powder by polyol method and its oxidation product. Mater Sci Eng B 122:222–225

Zhang Z, Sun X, Dresselhaus MS, Ying JY, Heremans J (2000) Electronic transport properties of single crystal bismuth nano-wire arrays. Phys Rev B 61:4850–4861