European Journal of Pharmaceutical Sciences 66 (2015) 123–137

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Design, synthesis and evaluation of antimalarial potential of polyphosphazene linked combination therapy of primaquine and dihydroartemisinin

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ARTICLE INFO

Article history:
Received 31 July 2014
Received in revised form 6 September 2014
Accepted 28 September 2014
Available online 12 October 2014

Keywords:
Resistant malaria
Substituted polyphosphazenes
Primaquine
Dihydroartemisinin
Nanoparticles

ABSTRACT

Various polymer drug conjugates (**13–16**) such as primaquine and dihydroartemisinin conjugated 2-propoxy substituted polyphosphazenes (**13**), primaquine and dihydroartemisinin conjugated 4-acetamidophenoxy substituted polyphosphazenes (**14**), primaquine and dihydroartemisinin conjugated 4-formyl substituted polyphosphazenes (**15**) and primaquine and dihydroartemisinin conjugated 4-aminoethylbenzoate substituted polyphosphazenes (**16**) were synthesized using substituted polyphosphazenes as polymer and primaquine and dihydroartemisinin as combination antimalarial pharmacophores and formulated to nanoparticles to achieve novel controlled combined drug delivery approach for radical cure of malaria. The polymeric backbone was suitably substituted to impart different physicochemical properties. The polymer-drug conjugates were characterized by IR, ^{1}H NMR, ^{31}P NMR and their molecular weights were determined by Gel Permeation Chromatography. The thermal properties of the conjugates (**13–16**) were studied by DSC and TGA. The conjugates (**13–16**) were then formulated to nanoparticles formulations to increase their uptake by hepatocytes and to achieve targeted drug delivery. The nanoparticle formulations were characterized by Zeta Sizer and their morphology were studied by TEM (Transmission Electron Microscopy) imaging. The nanoparticles formulations exhibited biphasic *in vitro* drug release profile, the initial burst release followed by a sustained release owing to the non-fickian diffusion during first step release and fickian diffusion during second step release. *In vivo* antimalarial efficacy was tested using *Plasmodium berghei* (NK65 resistant strain) infected swiss albino mice at different doses. The combination therapy exhibited promising antimalarial efficacy at lower doses in comparison to the standard drug combination. Further, this combination therapy provided protection over 35 days without any recrudescence, thus proving to be effective against resistant malaria. The study provides an alternative combination regimen found to be effective in the treatment of resistant malaria.

1. Introduction

Malaria is a disease caused by parasite of the genus *Plasmodium* and it is transmitted *via* the bites of infected female Anopheles mosquitoes. *Plasmodium falciparum* and *Plasmodium vivax* are the rampant species, but the former is the most lethal. Malaria is the most fatal human parasitic infection. At present, there are estimated 250 million cases of malaria world-wide. The majority of cases (86%) is reported in the African region, followed by the South-East Asia (9%) and Eastern Mediterranean regions (3%). There were estimated 0.881 million deaths globally in 2006, of which 90% were in the African province and 4% in each of the South-East Asia and the Eastern Mediterranean regions (World Malaria Report, 2008). The individual most at risk of significant morbidity and transience on account of malaria are the children under the age of 5 years and the pregnant women (Ashley et al., 2006; Lalloo et al., 2006). The preface of chloroquine (**1**) in the 1940s had a marvellous impact on health worldwide; however, today resistance to the drug has been observed in every region where *P. falciparum* occurs (Wongsrichanalai et al., 2002). The chloroquine (**1**) resistance has spread across all of Sub-Saharan Africa.

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http://dx.doi.org/10.1016/j.ejps.2014.09.023
0928-0987/

As a result, many countries switched their first-line antimalarial drugs to sulfadoxine (**2**) and pyrimethamine (**3**) combination therapy. However, resistance to sulfadoxine-pyrimethamine (SP) grew and spread rapidly, particularly in Southeast Asia, South America (White, 1992) and in recent times, covering many areas of Africa (Sibley et al., 2001). The ever-increasing spread of malaria in conjunction with the emergence of drug resistance against conventional drugs has put enormous pressure on public health systems to develop and launch new antimalarial drug remedy (Ridley, 2002). Several attempts have been accomplished to manufacture and appraise analogues of primaquine (**4**) in order to explore the compounds that would be more efficacious or less toxic. The addition of an amino acid or peptide residue to the primary amino group protected Primaquine against the aforementioned metabolic process and led to a considerable augmentation in antimalarial potential (Jain et al., 2004). Most of these derivatives are rapidly hydrolyzed to primaquine by *aminopeptidases* and *endopeptidases*, regardless of the improved activity/toxicity ratio (Borissova et al., 1995). Further, the coupling of primaquine covalently to lysosomotropic drug carriers–polyacryl starch microparticles have shown significant improvement in pharmacological activity of primaquine *in vivo* (Stjarnkvist, 1993). However, the conjugation of drugs to polymeric carriers to form macromolecular prodrugs is considered a useful approach to improve drug solubility, stability and prolong drug release, reduce doses, dosing intervals and drug toxicity or to accomplish targetability (Duncan, 2002). Other drug delivery systems described in literature include an ethyl cellulose-based transdermal therapeutic system (Mayorga et al., 1997). One of our co-author has contributed towards the development of implants for chloroquine (**1**) using biodegradable polymers, gelatin and cross-linked gelatin. The implants were evaluated for physicochemical properties, *in vitro* drug release study and pharmacokinetics. The results complied with optimum drug release conforming to the prerequisite of a long term implant for 7 days (Murthy et al., 2001). Their other contribution is reported to the artemether (**8**) loaded lipid nanoparticles developed by modified thin-film hydration which were found to be safe and more effective with respect to antimalarial potential when compared with standard and marketed formulations (Aditya et al., 2010a,b).

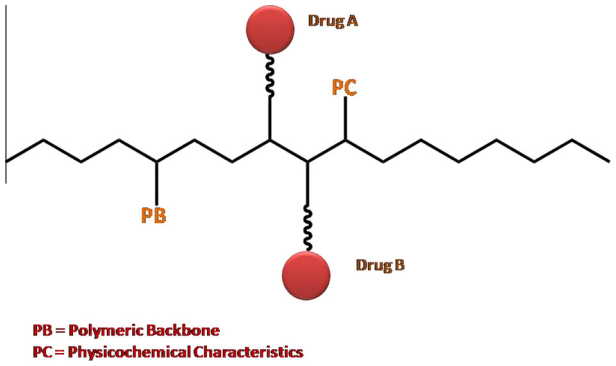

Fig. 1. Technical approach for designing polymer linked combination drug therapy.

The use of two antimalarial drugs concurrently, in particular when the drugs have different mechanisms of action, has the potential for inhibiting the development of resistance to either of the components. The efficacy of a combination of a 4-aminoquinoline drug either chloroquine (**1**) or amodiaquine (**5**) with sulfadoxine/pyrimethamine (SP) has been well established (McIntosh and Greenwood, 1998). The results revealed that the addition of either chloroquine (**1**) or amodiaquine to SP marginally improved parasitological clearance (compared with SP alone). Another combination therapy approach involves combining an artemisinin derivative with other, longer half-life antimalarials. Example of therapeutic combinations of mefloquine (**6**) and artemisinins (**7–11**) offer the only consistent treatment for even uncomplicated malaria, due to the development and pervasiveness of multi-drug resistant falciparum malaria. This combination therapy has been proven to be responsible for inhibiting amplification of drug resistance and for decreased malaria transmission levels in South-East Asia (White et al., 1999). Recently, Capela et al. designed, synthesized and evaluated the primaquine-artemisinin hybrids as multistage antimalarial strategy based upon the covalent combination of molecules acting on different stages of the parasite life cycle (Capela et al., 2011).

Therefore, taking into consideration the emergence of drug-resistant strains of *P. falciparum*, and prospective advantages of combination therapy, it was considered of interest to attach two antimalarial drugs with different mechanism of actions as combination therapy to biodegradable polymeric backbone by covalent bond so that the emergence of drug-resistant strains could be avoided.

Polyphosphazenes are polymers having an inorganic backbone and composed of altering nitrogen and phosphorus atoms linked by alternating single and double bonds with two substituents at each phosphorus atom. These are the most versatile inorganic polymers because a wide variety of substituents can be attached

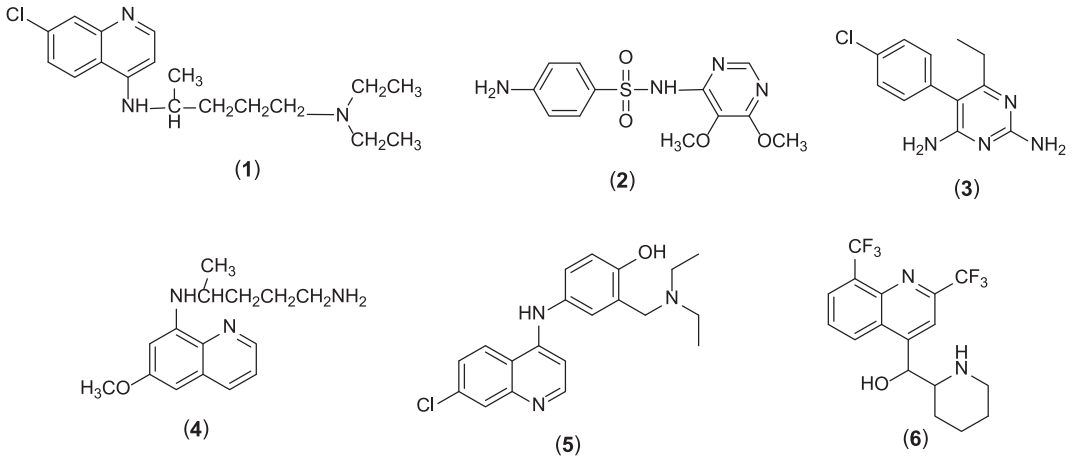

Fig. 2. Chemical structures of quinoline based and miscellaneous antimalarial drugs: chloroquine (**1**); sulfadoxine (**2**); pyrimethamine (**3**); primaquine (**4**); amodiaquine (**5**); mefloquine (**6**).