ANNEXURE I

Animal Ethical Certificate

J.S.S. College of Pharmacy, Ootacamund, Tamil Nadu, India.
Committee for the Purpose of control and Supervision of Experiments on
Animals (CPCSEA)
Institutional Animal Ethics committee (IAEC)

CERTIFICATE

Title of the Project: Novel drug targeting approach for management of
Alzheimer's disease.

Proposal Number: JSSCP/IAEC/PH.D/PH.CEUTICS/03/2013-13
Date received after modification (if any):
Date received after second modification: 20.11.2012
Approval date: 24.11.2012
Animals: Wild rats/ Albino mice
Rabbits / Guinea pigs
☑ Male/Female
No. of animals sanctioned: 56 RATS
Expiry date (Termination of the Project): 2 MONTHS

Name of IAEC/CPCSEA chairperson: Dr. K. Elango
(Principal)

Signature of Chairperson
Date: 24.11.2012

Chairperson

Institutional Animal Ethics Committee
JSS College of Pharmacy
Rooklands, Ooty 643001
J.S.S. College of Pharmacy, Ootacamund, Tamil Nadu, India.
Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA)
Institutional Animal Ethics Committee (IAEC)

CERTIFICATE

Title of the Project: Novel Drug Targeting Approach for management of Alzheimer's disease

Proposal Number: JSSCP/IAEC/PH.D/PH.AEETICS/01/2013-14

Date received after modification (if any):

Date received after second modification: 28.08.2013

Approval date: 28.08.2013

Animals: Wistar Rats

Male/Female

No. of animals sanctioned: 180

Expiry date (Termination of the Project): 30 Days

Name of IAEC/CPCSEA chairperson: Dr. K. Elango

[Signature]

Date: 30.08.2013

Chairperson
Institutional Animal Ethics Committee
JSS College of Pharmacy
Rocklands, Ooty-643001
ANNEXURE II
List of Awards, Publications, Presentations and Patent

AWARDS

**INSPIRE Fellowship** from Department of Science and Technology (DST), Government of India, New Delhi for pursuing doctoral research.

**Travel Grant** from DST for attending ASME 2nd Global Congress on NanoEngineering for Medicine and Biology - NEMB2013 held at Boston, USA from February 4-6, 2013.

**Travel Grant** from Indian Council of Medical Research (ICMR), New Delhi for attending Third International Conference on Multifunctional, Hybrids and Nanomaterials held at Sorrento, Italy from March 3-7, 2013.

**Travel Grant** from Council of Scientific and Industrial Research (CSIR), New Delhi and JSS University, Mysore for attending Alzheimer’s Association International Conference (AAIC) held at Boston, USA from July 13-18, 2013.

LIST OF PUBLICATIONS


1 First author/equal authorship
POSTER PRESENTATIONS

1. ‘Nanostructured Lipid Carriers for Intranasal Delivery for Improved Brain Targeting in Alzheimer’s Disease’ in ASME 2013 2nd Global Congress on NanoEngineering for Medicine and Biology-NEMB2013 organized by ASME in association with Harvard University and Massachusetts Institute of Technology at Boston, USA from February 4-6, 2013.


3. ‘Delivery of Neuroprotective Phenol to Brain via an Intranasal Route for Management of Alzheimer’s Disease’ in Alzheimer’s Association International Conference (AAIC) organized by Alzheimer’s Association held at Boston, USA from July 13-18, 2013. (Published in Alzheimer’s and Dementia, Vol.9 (4), July 2013, P299; IF 14.483)

PATENT

Application Filed
No. DST/INSPIRE Fellowship/2010

Date: 26 November 2010

Subject: Award of INSPIRE Fellowship to the Research Students [IF10316]

Dear Sumeet Sood,

The Government of India has launched a unique Scheme "Innovation in Science Pursuit for Inspired Research (INSPIRE)" with several components. INSPIRE Fellowship provides fellowship in Basic and Applied Sciences. I am pleased that you have been Selected for the award of INSPIRE Fellowship.

The value of the Fellowship will be at Par with the Junior Research Fellowship (JRF)/Senior Research Fellowship (SRF) of Government of India along with a Contingency grant. The Fellowship shall be available to you for a period of five years or completion of your doctoral (PhD) program, whichever is earlier. The Terms & Conditions of the INSPIRE Fellowship are presented in the website: www.inspire-dst.gov.in.

To
Mr. Sumeet Sood
Research Scholar, Dept. of Pharmaceutics,
J.S.S. College of Pharmacy, Rocklands, Post Box No. 20
Ooty-643001, Tamil Nadu
Science and Engineering Research Board (SERB)
(A Statutory body under Department of Science & Technology, Government of India)

SR/ITS/4608/2012-2013

To,

Sh. Sumeet Sood
D/o Pharmaceutics
JSS College of Pharmacy
Octacumund-643001 (Tamil Nadu)

Sub.: Financial Assistance to Sh. Sumeet Sood for participating in ASME 2013, 2nd Global Congress on NanoEngg. for Medicine and Biology to be held from 04/02/13 To 06/02/13 in U S A

Sir / Madam

We are happy to inform you that your application seeking financial grant to attend the above mentioned international scientific event has been recommended for support by the Science and Engineering Research Board (SERB). We will provide to and fro economic class air-fare by the shortest route, airport tax, visa fees and registration fees. We hope this support will provide you an opportunity to interact with leading international experts in the area. The support, however, is subject to the following conditions.

1. You should not have received financial support during last three years under this scheme.
2. The air tickets are to be booked in economic class by the shortest route in a National Carrier, i.e., Air India. For Travel to station not connected by Air India, you may travel by Air India to the hub/point closest to their eventual destination, beyond which you may utilize the services of another airline which should also preferably be an alliance partner of Air India. If you are traveling by Private Airline because of non-availability of tickets or any other reason, you are requested to seek relaxation from the Ministry of Civil Aviation. The Contact details for obtaining relaxation are

   (1) Shri P.N. Sukul, Joint Secretary
       Ministry of Civil Aviation
       Rajiv Gandhi Bhawan, New Delhi
       FAX 25655839, E-mail: sukulpn@nic.in

   (2) Shri S.K. Chhikara, Under Secretary
       Ministry of Civil Aviation
       Rajiv Gandhi Bhawan, New Delhi
       FAX 24651132, E-mail: chhikara.sk@nic.in

   You are advised to attach a copy of permission letter from Ministry of Civil Aviation for travel by private airlines while claiming the reimbursement. Without this permission letter, it will not be possible to reimburse the travel grant. However, during the Air India Strike Period, you can travel by Private Airlines till the service of Air India became normal.
3. E-ticket is acceptable provided the amount of the fare is clearly reflected on the ticket.
4. You will submit tour report and other documents in the enclosed proforma within 30 days of your return to India.
5. The claim-sheet along with all documents must be tagged/stapled properly before sending it to the Board.
   Institute/University Accounts Details should be signed by the competitive authority of the Institute/ University and Certified by Authorized Official of the Bank.
6. We will reimburse the grant after deducting the support received from any other sources, if any.
7. All other expenses such as per diem, taxi fare, bus fare etc. will not be reimbursed by the Department.
8. You have to make your own arrangements for foreign exchange required for the purpose.
9. You will not be treated as a delegate sponsored by the Government of India.

Based on this offer letter, your Institute may consider advancing necessary funds to enable you to attend the above event. We request you to intimate to us within two weeks, if you are not availing this offer.

With kind regards,

Your's Faithfully,

(Praveen Kumar S.)
Scientist – D

Encl: Claim Sheet
SANDHYA DIWAKAR
Scientist- E

Sumeet Sood,
Research Scholar,
Pharmaceutics,
JSS College of Pharmacy,
Rocklands, Ootacamund, Tamilnadu-643001


Dear Sir/Madam,

I am glad to inform you that Director General, ICMR, based on the recommendation of Expert Committee, has sanctioned a sum of Rs. 96,660/- (Rupees ninety six thousand six hundred sixty eight only) to you towards air fare, Registration & Visa fee (The air tickets are to be booked in economic class in a National Carrier i.e. Air India) to attend international conference/workshop/training.

If you are willing to avail the assistance, you may convey your acceptance within 15 days of issue of this communication, failing which it will be assumed that you are not interested to avail the grant. In the event of your not being able to utilize this amount for various reasons even after confirming your acceptance, please inform us immediately for necessary action at our end.

We have following comments to make:

The actual amount will be reimbursed after your return from the conference and receiving the required travel documents. Please find enclosed herewith accounts proforma in which you will have to submit your claim along with a copy of:

- Award letter
- Participation certificate and copy of presented paper in the proceedings/abstract book.
- Participation report, air ticket showing air fare and boarding pass original copy only (from Air India only as per Government orders)
- Award letter from other agencies.
- Any other relevant documents

You are requested to produce the original bills/vouchers. The claim should be forwarded to us through competent authority and should reach this office within one month after the completion of the scientific conference/workshop/training.

The financial assistance is governed by the terms and conditions as mentioned in enclosed form.

Yours faithfully,

SANDHYA DIWAKAR

For Director General ICMR
sandyadiwakar@yahoo.com
011- 26589287 (o)

Copy to: Principal, JSS College of Pharmacy, Rocklands, Ooty, Tamilnadu-643001.

Note: Journey by other than National carrier is not permissible under the Govt. of India norms. For travel to stations not connected by Air India, Officials may travel by Air India to the point closest to their eventual destination beyond which they may utilize the services of another airline, which should also preferably be an alliance partner of the national carrier (Air India). A certificate to this office may be obtained from Air India.
H R Grover
Scientist

Ref No. TG/7946/13-HRD
June 17, 2013

Mr Sumeet Sood
Research Scholar
c/o Dr. K.Gowthamarajan, Dept. of Pharmaceutics
J.S.S.College of Pharmacy
Ootacamund - 643 001

SUBJECT: CSIR Foreign Travel Grant

Dear Sir / Madam

With reference to your application on the aforesaid subject, we are happy to inform that the Director General, CSIR has been pleased to sanction foreign travel grant to enable you to attend and present your paper at the Alzheimers Association International Conference, USA during 13 Jul 2013 to 18 Jul 2013 subject to the following conditions:

1. The CSIR Foreign Travel Grant is limited to Full Air Fare only payable in Indian Rupees only. The journey should be strictly performed by the shortest route in excursion economy class by Air India only. Tickets should be purchased directly from the Airlines (at booking counters/Website of Airlines) or by utilizing the services of Authorized Travel Agents viz. M/s Balmer Lawrie & Company, M/s Ashok Travels & Tours as warranted under Govt. of India orders in this subject. Travel by Air India is mandatory. Fare will not be reimbursed if you travel by other airlines. In case of deviation because of operational or other reasons or on account of non-availability, relaxation/permission may be obtained from Under Secretary, Ministry of Civil Aviation, Rajiv Gandhi Bhawan Safdarjung Airport New Delhi 110003.

2. The grant should be claimed by filling-in the Tour report* & Grant-in-Aid bill* proforma (in duplicate) along with the counter foil of original boarding pass, original cash receipt/ e-ticket and certificate of attending the conference from the organizers. The grant should be claimed within one month of return from abroad forwarded through his/her Supervisor/Head of the Institution.

3. One reprint of the research paper presented at the Conference/Symposium etc. should be sent to CSIR, invariably after its publication.

4. Please communicate your acceptance of this grant immediately by email only, failing which it will be presumed that you do not need support from CSIR.

Yours sincerely,

(H R Grover)

Copy to:-

i) Principal
J.S.S.College of Pharmacy
Rocklands, Ootacamund - 643 001

ii) Audit (EMR)

* Note: Tour report & Grant-in-Aid Bill Proforma may be downloaded from our website http://csirhrdg.res.in
Intranasal therapeutic strategies for management of Alzheimer’s disease

Sumeet Sood*, Kunal Jain*, and K. Gowthamarajan

Department of Pharmaceutics, J.S.S. College of Pharmacy, Udhagamandalam, Tamil Nadu, India

Abstract

Alzheimer’s disease (AD) is a chronic and progressive age-related irreversible neurodegenerative disorder that represents 70% of all dementia with 35 million cases worldwide. Successful treatment strategies for AD have so far been limited, and present therapy is based on cholinergic replacement therapy and inhibiting glutamate excitotoxicity. In this context, role of neuroprotective drugs has generated considerable interest in management of AD. Recently, direct intranasal (IN) delivery of drug moieties to the central nervous system (CNS) has emerged as a therapeutically viable alternative to oral and parenteral routes. IN delivery bypasses the blood–brain barrier by delivering and targeting drugs to the CNS along the olfactory and trigeminal neural pathways which are in direct contact with both the environment and the CNS. In an attempt to understand how neurotherapeutics/nanoparticulate delivery systems can be transported from the nose to the CNS, the present review sets out to discuss the mechanism of transport from nose to brain. The aim of this review is to discuss and summarize the latest findings of some of the major studies on IN drug delivery in AD models, with a focus on the potential efficacy of neuroprotective treatments.

Keywords

Alzheimer’s disease, dementia, intranasal, nanoparticles, neuroprotective

Introduction

Alzheimer’s disease (AD) is a chronic and progressive age-related neurodegenerative disorder that represents 70% of all dementia with 36 million cases worldwide and projections suggest that these may increase to 115 million by 2050 [1,2]. It is characterized by accumulation of β-amyloid (Aβ), senile plaques, neurofibrillary tangles (NFTs), cognitive and memory impairments; eventually progressing to physical impairment and death [3]. Cerebrovascular pathology, including cerebral amyloid angiopathy is observed in AD patients [4]. The number of people suffering from AD has been increasing exponentially with one new case every 4 min. The cognitive decline associated with AD drastically affects the social and behavioural skills of the people living with the disease. Notwithstanding the social impact, however, AD also imparts great financial burden on patients, families and communities as a whole [5]. As such, the disease poses heavy economic and societal burden, with associated annual cost of care over $100 billion [6].

There are several limitations associated with present therapy and intranasal (IN) strategy seems to be promising route for delivery of drugs to brain. In an attempt to understand how neurotherapeutics/nanoparticulate delivery systems can be transported from the nose to central nervous system (CNS), the relevant mechanisms of nose to brain transport are discussed. The overall aim of this review is to discuss and summarize the latest findings of some of the major studies that convincingly show the transport of drugs into the CNS following IN route in AD models, with a focus on the potential efficacy of neuroprotective treatments.

Genetics of AD

On the basis of genetic etiology, AD can be classified as familial AD (FAD) and sporadic AD (SAD). The inheritance of AD has been explained by genetic analysis of some rare cases of early onset FAD [7]. FAD represents <1% of all AD cases and is caused by mutations in app, ps1 and ps2 genes responsible for expression of amyloid precursor protein (APP), presenilin-1 and -2 (PS-1 and PS-2) proteins. They are located on chromosome 21, 14 and 1, respectively [8]. SAD occurs at >65 years of age and represents 99% of AD cases. In majority of these cases, there were no mutations in genes involved in FAD [9]. Olson and co-workers have found a gene on chromosome 20p to be involved in SAD [10,11]. Mutations and polymorphism in multiple genes contribute to SAD pathogenesis along with non-genetic factors [7]. Elevated Aβ levels in brain generate neurotoxic Aβ oligomers which may disrupt normal brain function or aggregate to form plaques which represent a major pathological step in progression of AD. In addition, polymorphism in genes for apolipoprotein E (apoE), α-2 microglobulin, very low
Optimization of curcumin nanoemulsion for intranasal delivery using design of experiment and its toxicity assessment

Sumeet Sood *, Kunal Jain, K. Gowthamarajan *
Department of Pharmaceutics, J.S.S. College of Pharmacy, Udhagamandalam, Tamilnadu 643001, India

A R T I C L E   I N F O
Article history:
Received 12 June 2013
Received in revised form 9 September 2013
Accepted 14 September 2013
Available online 21 September 2013

Keywords:
Box–Behnken design
Curcumin
Intranasal
Mucoadhesive
Nanoemulsion

A B S T R A C T
The objective of the study was to optimize curcumin nanoemulsion for intranasal delivery using design of experiment. Box–Behnken design was constructed using oil, surfactant and co-surfactant concentration as independent variables and their affect on response y1 (globule size) and y2 (zeta potential) were studied. The ANOVA test identified the significant factors that affected the responses. For globule size, percentage of oil, surfactant and co-surfactant were identified as significant model terms whereas for zeta potential, oil and co-surfactant were found to be significant. Critical factors affecting the responses were identified using perturbation and contour plots. The derived polynomial equation and contour graph aid in predicting the values of selected independent variables for preparation of optimum nanoemulsion with desired properties. Further, 2^4 factorial design was used to study influence of chitosan on particle size and zeta potential. The formulated developments did not show any toxicity and were safe for intranasal delivery for brain targeting. In vitro diffusion studies revealed that nanoemulsions had a significantly higher release compared to drug solution. Ex vivo diffusion studies were carried out using sheep nasal mucosa fixed onto Franz diffusion cells. Mucoadhesive nanoemulsion showed higher flux and permeation across sheep nasal mucosa.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction
Curcumin (diferuloyl methane) is a phenolic phytochemical obtained from rhizome of herb Curcuma longa L, commonly known as turmeric [1]. It has been found to exert beneficial effects on experimental models of Alzheimer’s disease (AD) [2]. In vitro studies have shown that curcumin inhibit amyloid–β (Aβ) aggregation and Aβ induced inflammation [3–5]. Oral administration of curcumin in AD animal models has been found to inhibit Aβ deposition, Aβ oligomerization and tau phosphorylation in brain [3,6,7]. Curcumin has been found to decrease Aβ related inflammation and Aβ burden in amyloid precursor protein (APP) transgenic mice [6]. It also enhances activity of glutathione-S-transferase and inhibits nuclear factor κ beta (NFκβ). Activation of NFκβ increases the transcription of various inflammatory mediators [8,9]. Further, curcumin has been found to improve memory and cognitive deficits in rats [10]. Inspite of being a ‘wonder molecule’ the therapeutic efficacy of curcumin is limited by poor aqueous solubility, chemical instability in alkaline medium, rapid metabolism and poor absorption from gastrointestinal tract [11].

Delivery of drugs to brain for treatment of central nervous system (CNS) disorders is hindered by restrictive barriers like blood–brain barrier (BBB) and blood–cerebrospinal fluid barrier [12]. One of the promising strategy to deliver neurotherapeutics to brain is intranasal delivery. It is a non-invasive technique for bypassing BBB and ensures direct delivery to CNS [13]. Many studies have indicated direct transport of drugs to cerebrospinal fluid and various parts of brain [14,15]. However, intranasal delivery is limited by rapid mucociliary clearance and poor nasal permeability of nasally applied drugs [13]. Alternative approaches that have been utilized to overcome these problems are use of chemical penetration enhancers and colloidal drug delivery systems (nanoemulsions, liposomes and nanoparticles) [16]. Most efforts in intranasal delivery have been focused on increasing the drug absorption, enhancing the nasal retention time and stability of the drug with the final goal of improving the therapeutic outcome. For treatment of CNS disorders like AD, these attempts include the design of mucoadhesive carrier system with improved drug delivery properties to the nasal cavity. Among these mucoadhesive nanoemulsions have been studied extensively. Nanoemulsions are heterogeneous systems consisting of fine oil-in-water dispersions stabilized by surfactant molecules. Moreover, they are kinetically stable without any apparent flocculation or
Lipid Nanocarriers and Molecular Targets for Malaria Chemotherapy

Kunal Jain*†, Sumeet Sood* and K. Gowthamarajan*

Department of Pharmaceutics, J.S.S. College of Pharmacy, Udhagamandalam, Tamilnadu-643001, India

Abstract: Malaria is the most serious tropical disease of humankind and a cause of much debilitating and morbidity throughout the world especially in endemic areas like India and Africa. The development of drug resistance may be due to insufficient drug concentration in presence of high parasite load. In addition, the present pharmaceutical dosage forms are ineffective thereby necessitating the development of novel dosage forms which are effective, safe and affordable to underprivileged population of the developing world. The rapid advancement of nanotechnology has raised the possibility of using lipid nanocarriers that interact within biological environment for treatment of infectious diseases. Thus, lipid based nano-delivery systems offer a platform to formulate old and toxic antimalarial drugs thereby modifying their pharmacokinetic profile, biodistribution and targetability. Further, there is a need to develop new chemotherapy based approaches for inhibiting the parasite-specific metabolic pathways. The present review highlights the advances in lipid nanocarriers and putative molecular targets for antimalarial chemotherapy.

Keywords: Anti-malarial, lipid, malaria, molecular targets, nanocarriers, Plasmodium.

INTRODUCTION

Malaria is the most serious tropical disease of humankind and a cause of much debilitating and morbidity throughout the world especially in endemic areas like India and Africa. It is responsible for 225 million clinical cases and 7, 81,000 deaths per year worldwide of which 91% are in the African region. About 85% of these deaths were in children under 5 years of age [1]. Besides acquired immune deficiency syndrome (AIDS) and tuberculosis, malaria is one of the world’s biggest public health problem. A total of US $ 38-45 billion will be spent from 2006-2015 for the diagnosis and treatment of malaria [2], mainly in underdeveloped countries which are most affected by this deadly disease [3]. Malaria is commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development [4]. In humans, malaria is caused by five distinct blood-borne parasite species: Plasmodium falciparum, P vivax, P. ovale and P. malariae and P. knowlesi [5].

Amongst these, the most severe malaria is caused by P.falciparum which is responsible for almost all malaria related deaths worldwide [6]. It is most dangerous in children because of their developing and immature immune system and in pregnant women due to recoverable loss of immunity during pregnancy [7]. The disease targets liver and red blood cells and has a latent phase in the liver which can result in reoccurrence after many years, even after successful antimalarial treatment [8]. The present therapy is associated with frequent failures such as complexity of the parasite life cycle, development of drug resistance and difficulty in controlling transmission of malarial parasite [9, 10]. The development of drug resistance may be due to insufficient drug concentration in presence of high parasite load. In addition, the present pharmaceutical dosage forms are ineffective thereby necessitating the development of novel dosage forms which are effective, safe and affordable to underprivileged population of the developing world and impose less financial burden on their governments. Furthermore, vaccination against malarial parasite has not yielded promising results due to the fact that it does not illicit a pronounced immune response.

The present review focuses on the application of lipid nanoparticles as a promising strategy for malaria treatment. The emphasis is particularly laid on liposomes, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLC), nanoemulsions (NE) and microemulsions (ME). In addition, we have discussed in detail about novel molecular targets which could be utilized in designing new antimalarials.

LIFE CYCLE OF PLASMODIUM

Life cycle of Plasmodium sp. is quite complex involving two host’s viz. man and mosquito. When an infected mosquito bites a human host, motile sporozoites in the mosquito saliva enter the blood stream and migrate to liver. Within 30 minutes, the sporozoites rapidly infect hepatocytes multiplying asexually and asymptomatically for a period of 1-2 weeks (exocytocytic stage). Later they produce thousands of merozoites, which following rupture of their host cells escape into the blood, infect the red blood cells (RBC) and develop through the ring, trophozoites (growing) and schizont (dividing) stages. Within the RBC, the parasites multiply further, periodically breaking out of their hosts to invade fresh RBC (erythrocytic stage) and differentiate into male and female gametocytes that are capable of transmission to mosquitoes [11, 12]. If unchecked by protective immune responses or an antimalarial chemotherapy, successive cycles of erythrocytic growth gradually result in increasing...
Review article

Modulation of cerebral malaria by curcumin as an adjunctive therapy

Kunal Jain1,*, Sumeet Sood1, K. Gouthamarajan

Department of Pharmaceutics, J.S.S. College of Pharmacy, Udhagamandalam, Tamilnadu 643001, India

A R T I C L E   I N F O

Article history:
Received 12 January 2013
Accepted 21 March 2013
Available online 29 July 2013

Keywords:
Cerebral malaria
Curcumin
Adjunctive
Cognitive deficit

A B S T R A C T

Cerebral malaria is the most severe and rapidly fatal neurological complication of Plasmodium falciparum infection and responsible for more than two million deaths annually. The current therapy is inadequate in terms of reducing mortality or post-treatment symptoms such as neurological and cognitive deficits. The pathophysiology of cerebral malaria is quite complex and offers a variety of targets which remain to be exploited for better therapeutic outcome. The present review discusses on the pathophysiology of cerebral malaria with particular emphasis on scope and promises of curcumin as an adjunctive therapy to improve survival and overcome neurological deficits.

© 2013 Elsevier Editora Ltda. All rights reserved.

Introduction

Parasitic diseases have grown to become a health burden as around 30% of the world’s population experience parasitic infections.1 Among various parasitic infections, malaria is the most life-threatening disease and accounts for 225 million clinical cases and 781,000 deaths per year worldwide of which 91% are in the African region. Beside this background, it is reported that each year about 85% of deaths globally are in children under five years of age, mainly due to the immunological factors.2 A total of US$ 38-45 billion will be spent from 2006 to 2015 for the diagnosis and treatment of malaria, mainly in underdeveloped countries which are the most affected by this deadly disease.3 Cerebral malaria (CM) is the most severe and rapidly fatal neurological complication of Plasmodium falciparum infection and responsible for more than two million deaths annually in non-immune individuals characterized by impaired consciousness.4 This represents an enormous burden of disease, due to the high prevalence of infection. According to the World Health Organization (WHO), CM is defined as a clinical syndrome characterized by coma (inability to localize a painful stimulus) at least 1 h after termination of a seizure or correction of hypoglycaemia, presence of asexual forms of P. falciparum malaria parasites on peripheral blood smears and exclusion of other causes of encephalopathy.5

Till now, no effective vaccine is available against malaria because of the antigenic variation and complexity of parasite biology. The current therapy for management of CM is based on parenteral administration of either quinine or artesiminin derivatives such as artesunate and artemether. However, the emergence of drug resistance of P. falciparum to anti-malarials poses a serious challenge to malaria control. In addition, there are several non-parasitic events that contribute to
Optimization of artemether-loaded NLC for intranasal delivery using central composite design

Kunal Jain, Sumeet Sood, and Kuppusamy Gowthamarajan

J.S.S. College of Pharmacy, Department of Pharmaceutics, Rocklands, Udhagamandalam, Tamil Nadu, India

Abstract

The objective of the study was to optimize artemether-loaded nanostructured lipid carriers (ARM-NLC) for intranasal delivery using central composite design. ARM-NLC was prepared by microemulsion method with optimized formulation having particle size of 123.4 nm and zeta potential of -34.4 mV. Differential scanning calorimetry and powder X-ray diffraction studies confirmed that drug existed in amorphous form in NLC formulation. In vitro cytotoxicity assay using SVG p12 cell line and nasal histopathological studies on sheep nasal mucosa indicated that all the developed formulations were non-toxic and safe for intranasal administration. In vitro release studies revealed that ARM-NLC showed sustained release up to 96 h. Ex vivo diffusion studies using sheep nasal mucosa revealed that ARM-NLC had significantly lower flux compared to drug solution (ARM-SOL). Pharmacokinetic and brain uptake studies in Wistar rats showed significantly higher drug concentration in brain in animals treated intranasally (i.n.) with ARM-NLC. Brain to blood ratios for ARM-NLC (i.n.), ARM-SOL (i.n.) and ARM-SOL (i.v.) were 2.619, 1.642 and 0.260, respectively, at 0.5 h indicating direct nose to brain transport of ARM-NLC showed highest drug targeting efficiency and drug transport percentage of 27.16 and 64.02, respectively, which indicates ARM-NLC had better brain targeting efficiency compared to drug solution.

Introduction

Cerebral malaria (CM) is the most severe and rapidly fatal neuromorbidity complication of Plasmodium falciparum infection and responsible for more than 2 million deaths annually in nonimmune individual. This represents an enormous burden of disease, due to the high prevalence of infection (Jain et al., 2013a). It is characterized by impaired consciousness, seizures, hallucinations, severe metabolic acidosis, jaundice, renal failure and respiratory distress (Beales et al., 2000; Maidland & Newton, 2005). The underlying factors that are hallmark of cerebral malaria are sequestration and cytotoxicity of infected RBC, platelets, leukocytes; resetting, auto-agglutination, release of inflammatory cytokines, hypoxia and cerebral oedema. As a result of these central nervous system (CNS) complications, the disease may progress to unarousable coma and death (Newton et al., 2000).

Artemether (ARM) is oil soluble methyl ether of artemisinin effective against both chloroquine-resistant and chloroquine-sensitive strains of P. falciparum, as well as against Plasmodium vivax. It is also used in the management of CM (Medana & Turner, 2006). It contains sesquiterpene lactone rings with an endoperoxide bridge that is cleaved by an iron-dependent mechanism. It is a potent inhibitor of cysteine protease by virtue of its inhibition of hemoglobin formation as well as hemoglobin degradation (Klayman, 1985). It suffers from poor aqueous solubility and short half life usually between 3 and 5 h. Furthermore, oily intramuscular (i.m) injection of ARM for the treatment of CM is associated with pain on injection, erratic absorption and thus poor patient compliance. In addition, i.m. administration is not suited to deliver the drug to treat CM or when quick eradication of the malarial parasite is required (Aditya et al., 2010). Treatment of CM requires hospital admission, since it requires parenteral administration. This is a major limitation as hospitals are not accessible in all the endemic areas (Toutou et al., 2006). Hence to overcome these inherent drawbacks associated with the parenteral delivery of ARM, efforts are being undertaken to investigate alternative modes of antimalarial drug delivery to the brain. The conventional drug delivery system that releases the drug into systemic circulation fails to deliver drugs effectively to brain and is therefore not very useful in treating CM. Therefore, there is need for a patient compliant method to deliver ARM to the brain in a better and effective way.

In the recent years, intranasal (i.n) administration has received a great deal of attention as a convenient, reliable and an acceptable alternative to parenteral administration of various drugs to target brain directly via the olfactory neurons. The nasal route of drug administration provides a route of entry to the brain that circumvents the blood-brain barrier (BBB) and this neuronal connection constitutes a...