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Research Article

Design and in vitro evaluation of nanoemulsion for nasal delivery of artemether

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ABSTRACT

Nanoemulsion system with Tween 80, campul PG8 as surfactant and ethyl oleate as oil was developed for intranasal delivery of artemether. The selected nanoemulsion system is evaluated for particle size, zeta potential and stability. Ex vivo studies like permeation and histological examination were also carried out. The oil in water type nanoemulsion system was obtained containing 2:1 ratio of surfactant and co surfactant. The nanometric size of globule was retained even after 100 times dilution with water. The negative zeta potential reveals anionic charge on surface of globule. The ex vivo permeation of artemether across nasal mucosa is rapid. This improved permeation of artemether was result of presence of surfactant which reduces the interfacial tension at the mucosal surface nearly to zero. The enhanced permeation also explained in terms of large surface area of the nanoemulsion droplets. Histological examination of nasal mucosa did not detect any damage during in vitro permeation studies. This study points to the potential of nasal nanoemulsion in terms of ease of administration, improved permeation across the nasal mucosa and safety. This novel mode of administration may consider as an alternative to conventional treatment of cerebral malaria.

INTRODUCTION

Nanoemulsions are transparent or translucent dispersions, having the droplet size of less than 100 nm (the same droplet length-scale as microemulsions) with ultra low interfacial tension, large o/w interfacial areas and long-term physical stability Recently, much attention has been paid to the application of nanoemulsions as drug delivery systems, since nanoemulsions are thermodynamically stable and are formed spontaneously by simple mixing of the various components. The following main advantages have made these systems unique and, therefore, attracted much attention for their application in pharmaceutics and drug delivery. Higher solubilization capacity, compared to simple micellar solutions, can improve the solubility and bioavailability of hydrophobic compounds. Enormous increase in the interfacial area can influence the transport properties of the drug.

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Brownian motion can keep the droplets from creaming or sedimenting and eventually coalescing. Small droplet size prevents any flocculation, enabling the system to remain dispersed with no separation. Less surfactant concentration is required to prepare nanoemulsion, compared to microemulsions. Nanoemulsions are non-toxic and non-irritant and do not damage human and animal cells and hence are suitable for therapeutic purposes [1].

Malaria is an infectious disease caused by the Plasmodium genus of protozoan parasite. Malaria remains the world's most devastating human parasitic infection, afflicting more than 500 million people and causing from 1.7 million to 2.5 million deaths each year. Cerebral malaria is the most severe and life threatening complication of Plasmodium falciparum malaria and carries a case fatality rate of 5-40%, with most deaths occurring within the first 24hours [2,3]. In cases of severe malaria, due to vomiting and convulsions, oral medication is frequently not tolerated [4]. Therefore, efforts are being undertaken to investigate alternative modes of antimalarial drug delivery, such as rectal or transdermal administration [5,6]. Treatment of

CM, which is usually by i.v. injection, requires hospital admission [7]. This represents an additional problem, since hospitals are not easily and immediately accessible in all affected areas. In the present study we propose a novel treatment approach – intranasal administration of antimalarial drugs. One particular advantage of the nasal route is the simplicity of administration, allowing easy treatment following the first signs of illness [8]. Intranasal administration of artemether may facilitate prompt treatment following the first signs of malaria, preventing the development of CM. The drug chosen in this work was artemether (ARM) which is highly efficient against the blood stages of Plasmodium and against multi drug-resistant plasmodium falciparum. Artemether is poorly water soluble drug, by using the nanoemulsion system, an attempt was made to increase its solubility, thereby increasing its bioavailability. The aim of this current study was to develop an artemether containing nanoemulsion for nasal delivery that may be an alternative, effective and convenient administration method for drugs given by injection.

MATERIALS AND METHODS

Artemether (ARM) was received as a gift from Macleoids pharmaceuticals, Mumbai, India. Capmul PG 8 (Propylene Glycol Monocaprylate) was obtained as a gift sample from Abitec Corporation USA. Ethyl oleate was received as a kind gift from SD Fine chemicals, Mumbai. Tween 80 and ethanol was procured from Loba Chemie, Mumbai, India. All other reagents used were of analytical grade.

Preparation of Namoemulsion Formulation

Hydrophilic Surfactant (Tween 80) and lipophilic cosurfactant (Capmul PG 8) were mixed (Smix) in different volume ratios (1:1, 2:1, 3:1). These Smix ratios were chosen to reflect increasing concentrations of surfactant with respect to cosurfactant for detailed study of the phase diagrams in the nanoemulsion formation. Ethyl oleate optimized as an oil phase based on the solubility study. For each phase diagram, oil (ethyl oleate) and specific Smix ratio were mixed thoroughly in different volume ratios from 1:9 to 9:1 in different glass vials. Nine different combinations of oil and Smix (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) were made for the study to delineate the boundaries of phases precisely formed in the phase diagrams.

Ethyl oleate was blended with Tween 20 and Capmul PG 8 in fixed weight ratios (2:1). Artemether (2% w/w) was dispersed into the mixture of ethyl oleate and Tween 20 and Capmul PG 8 with a constant stirring until the mixture became clear. The mixture was gently shaken and kept at ambient temperature (25°C) to obtain a clear or translucent nanoemulsion.

Construction of Phase Diagrams

For convenience, the phase diagrams were constructed by drawing "water dilution lines" representing increasing water content and decreasing surfactant-co surfactant levels. The water was titrated along dilution lines drawn from the surfactant-co surfactant apex (100% surfactant-co surfactant) to the opposite oil side of the triangle. The line was arbitrarily denoted as the value of the line intersection with the oil scale. If turbidity appeared followed by a phase separation, the samples were considered to be biphasic. If clear and transparent mixtures were visualized after stirring, the samples were considered monophasic. The samples were marked as points in the phase diagram. The area covered by these points was considered to be the nanoemulsion region of existence [9]. Different formulations were selected from Fig.1 on the above-based criteria and were subjected to different thermodynamic stability tests.

Physicochemical evaluation of Artemether-loaded nano emulsions

Stability of nanoemulsions

The physical stability of nanoemulsions was evaluated by measuring particle size changes at designated time intervals after accelerated stress testing such as freeze thaw cycling [10].

Centrifugation

The nanoemulsion system was centrifuged at 3000 rpm for 15 minutes to determine whether the system shows signs of creaming or phase separation. The system was observed microscopically for appearance.

Drug content

The drug content of formulation was determined by UV spectrophotometric method. About 10 mg equivalent of artemether containing nanoemulsion was dissolved in 100ml of ethanol. From this stock solution, take 1 ml and dilute it in 10ml hydrochloric ethanol. The concentration of solution was found to be 1000 µg/ml. The drug content was estimated at 254nm.

Measurement of particle diameter and zeta potential

The mean particle diameter and polydispersity index (PI) of Artemether nanoemulsion were determined by photon correlation spectroscopy using particle size analyzer (ZS -90, Malvern Instruments, UK) at room temperature [11]. Prior to the measurement, nanoemulsion was diluted with filtered water to an adequate scattering intensity. The system was used in the auto-measuring mode. The laser diffraction particle diameter analysis data were evaluated using volume distribution to detect even a few large particles. The PI is a measure of the distribution of nanoparticle population. The zeta potential was determined using zeta sizer (ZS -90, Malvern Instruments, UK).Zeta potential measurements were performed after dilution with filtered distilled water at room temperature [12].

Ex-vivo Permeation Studies

Fresh nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Tissue samples were inserted in Franz diffusion cells displaying a permeation area of 0.785 cm2. About 16 ml of simulated nasal fluid (SNF) pH 6.6 at 370C± 0.5 0C was added to the acceptor chamber. To ensure oxygenation and agitation, a mixture of 95% O2 and 5% CO2 was bubbled through the system. After a pre-incubation time of 20 minutes, pure drug solution and formulation equivalent to 10 mg of artemether was placed in the donor chamber. At predetermined time points, 1-ml samples were withdrawn from the acceptor compartment, replacing the sampled volume with SNF pH 6.6 after each sampling, for a period of 5 hours. The samples withdrawn were filtered and used for analysis. Blank samples (without artemether) were run simultaneously throughout the experiment to check for any interference [13]. The amount of permeated drug was determined using a UV-visible spectrophotometer at 254 nm. Permeability coefficient (p) was calculated by the following formula:

$$P = \frac{\frac{dQ}{dt}}{C_0 \times A}$$

Where, dQ/dt is the flux or permeability rate (mg/h), C0 is the initial concentration in the donor compartment, and A is the effective surface area of nasal mucosa.

Histological examination of nasal mucosa

Sheep nasal mucosa obtained from a local abattoir within 2 hour of killing the animal was cleaned by washing with isotonic saline solution. 5 hours after applying the loaded nanoemulsion formulation, the nasal mucosa was fixed in 10% neutral carbonate buffered formalin solution, routinely processed and embedded in paraffin. Paraffin sections (7 µm) were cut on glass slides and stained with hematoxylin and eosin (HE). Sections were examined under a light microscope, to detect any damage to the tissue during in vitro permeation by a pathologist blinded to the study [14].

Accelerated stability studies

The nanoemulsion batches of optimized formulation were stored in stability chamber (Remi CHM-10S®) at 400C and 75% RH for 3 month and samples were evaluated for drug content.

RESULTS

Phase Diagram Construction

A pseudoternary phase diagram of the investigated quaternary system water/Tween 80 / Capmul PG8 / Ethyl oleate is presented in Fig 1. Formation of nano emulsion systems (the shaded area) was observed at room temperature. Phase behavior investigations of this system demonstrated the suitable approach to determining the water phase, oil phase, surfactant concentration, and co surfactant concentration with which the transparent, 1-phase low-viscous nano emulsion system was formed.

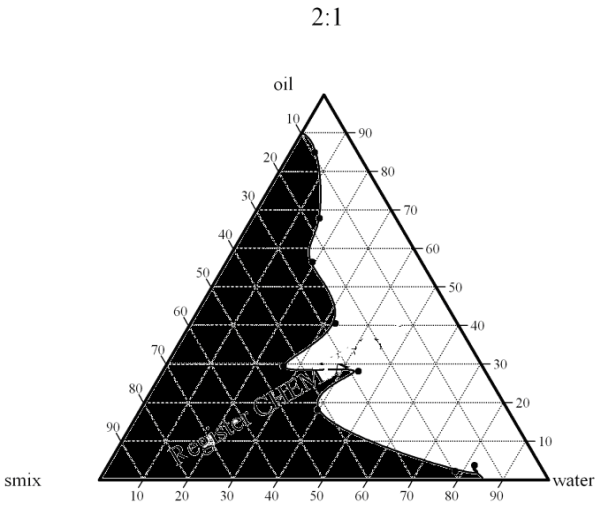

Figure 1: Phase diagrams of nanoemulsion containing ethyl oleate, Tween 80-Capmul PG 8(2:1) and water

The above figure represent the pseudo ternary phase diagrams for nanoemulsions systems along with the ratios of surfactant and co surfactant, as 1:1 and 2:1. Each of the vertices of triangle represents 100% of each of oil, water and surfactant and co surfactant mixture (Smix).

Physicochemical evaluation of nanoemulsion

The formulation withstands accelerated stress test as freeze thaw cycling confirms the thermodynamic stability of nanoemulsion. The nanoemulsion was also found to be stable on centrifugation at 3000 rpm for 15min. Nanoemulsion developed was clear and transparent, with pH 5.8 and drug content was found to be 96.98 %. The nanoemulsion has a globule size globule size 18.98 nm and polydispersity index of 0.317.This nanometric size range of particle was retained even after 100 times dilution with water, which proves systems compatibility with excess water. The mean globule diameter and the polydispersity of nanoemulsion are important parameters predicting the physical stability and in vivo fate of colloidal drug carrier such as nanoemulsion.The enhanced absorption may be explained in terms of the huge specific surface area of the nanoemulsion droplets. Zeta potential gives certain information about the surface charge properties and further the long-term physical stability of the nanoemulsions. The value of particle surface charge indicates the strength of the interactive force between particle and particle at the nano-surfaces, which is the basis to nanoemulsion stability at the macroscopic level. In order to obtain an electrostatically stabilized nanoemulsion, a minimum zeta potential of ± 30 mV is required. The zeta potential of nanoemulsion was -5.56mv Improved permeation of the artemether was result of presence of surfactant, which reduces the interfacial tension to nearly zero.

Ex vivo permeation study

Nanoemulsion formulation was subjected to ex vivo permeation studies using the sheep nasal mucosa. Ex vivo nasal mucosa permeability profile was shown in Fig. 2. The drug diffused at faster rate from nanoemulsion. The total percentage diffusion was much higher from the nanoemulsion system. The percent drug permeated after 5 h was found to be 92.68% from nanoemulsion.The permeability coefficient (P) was also calculated and found to be 1.482 mg/h. This higher rate of permeation of artemether from nanoemulsion was result of reduced particle size in presence of surfactant.

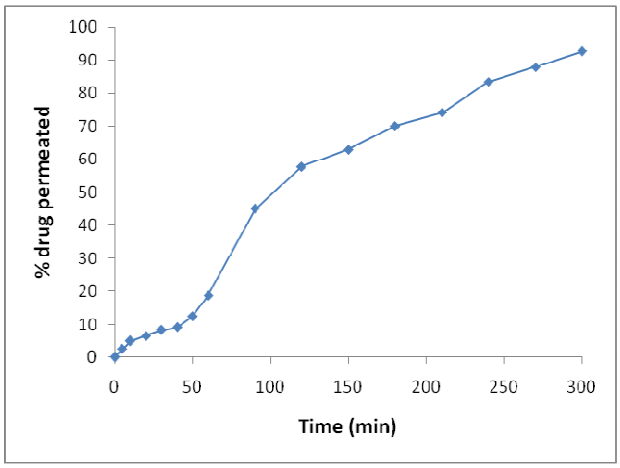

Figure 2: *Ex- vivo* permeation profile of ARM through sheep nasal mucosa

Histological examination of mucosa

The microscopic observations indicate that the optimized formulation has no significant effect on the microscopic structure of mucosa. As shown in Fig. 3, neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed after permeation of nanoemulsion. The epithelium layer was intact and there were no alterations in basal membrane and superficial part of submucosa as compared with SNF-treated mucosa. Thus, nanoemulsion formulations seem to be safe with respect to nasal administration.

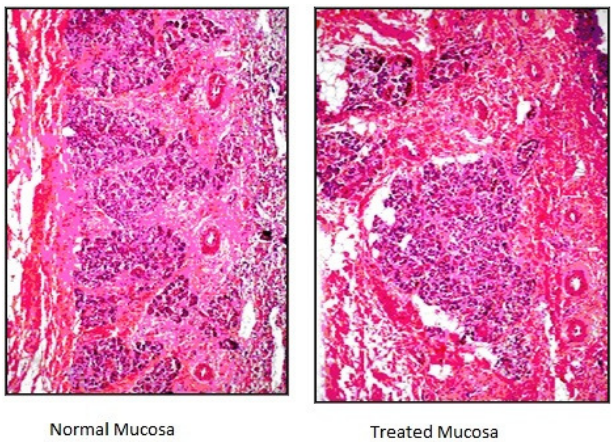

Figure 3: Histological photomicrograph of nasal mucosa

Accelerated stability studies

According to ICH guidelines, selected formulation was stored at 400C temperature and 75% relative humidity (RH) for a period of 3 months. Nanoemulsion remained clear and

transparent. Formulation was evaluated at periodical intervals of one month for drug content; the average drug content was remained relatively unchanged.

DISCUSSION

Over one million deaths each year are caused by malaria due to plasmodium falciparum infection. Artemether has been widely used drug for severe malaria. It has been considered as alternative to quinine as they have be shown to effective against multiple drug resistant malaria parasites. This is the most efficient antimalarial drug. However the oral mode of delivery of antimalarial drug is not optimized and sometimes cannot be used for the treatment of malaria. With this in mind; we developed nanoemulsion nasal drug delivery system of antimalarial drug artemether. This delivery mode could improve the efficiency of treatment, and overcome problem associated with oral delivery.

In this study nanoemulsion delivery system suitable for nasal administration was developed using GRAS (generally regarded as safe) excipients. The ex vivo permeation studies; reveals faster transport of drug across the nasal mucosa. Histological examination indicates safety of formulation toward nasal mucosa. Formulation found to be stable after exposure to accelerated conditions of temperature and humidity indicating that storage at specialized conditions is not required for the formulations.
Further studies shall focus on in vitro studies of formulation in suitable experimental models. Development of nasal drug delivery system encompasses through understanding of drug permeation potential. It is imperative to carry out extensive pharmacokinetic and pharmacodynamic studies to establish correlation if any, before establishing nasal delivery as an alternative.

CONCLUSIONS

Artemether containing nanoemulsion formulation was successfully prepared by spontaneous emulsification method (titration method) was feasible for nasal administration, and was expected to rapidly exert its antimalarial effect. The result of in vitro and ex vivo studies presented here lies in the possibility of substituting conventional antimalarial treatment methods with a novel drug administration mode, which may be useful in rapidly reducing the parasite bio-mass of an infected patient, thereby increasing the patient's chance of recovery while decreasing treatment costs. However, extensive pharmacokinetics and pharmocodynamic studies are required to establish a correlation, if any, before establishing artemether nanoemulsion nasal delivery as an alternative.

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REFERENCES

[1] Reza Aboofazeli Nanometric-Scaled Emulsions (Nanoemulsions) Iranian J of Pharm Res 2010; 9 (4): 325-326.
[2] War rell D, M E M, Beales P. Severe and complicated malaria. Trans R Soc Trop Med Hyg.1990; 84:1-65.
[3] D 'Alessandro U. Treating severe and complicated malaria. BMJ 2004; 328:155.
[4] WHO, severe falciparum malaria. Trans. R. Soc. Trop. Med. Hyg. 2000; 94: 1–90.
[5] Lin, A.J., Ager Jr., A.L., Klayman, D.L., Antimalarial activity of dihydroartemisinin derivatives by transdermal application. Am. J. Trop. Med. Hyg. 1994; 50: 777–783.
[6] Karunajeewa, H.A., Kemiki, A., Alpers, M.P., Lorry, K., Batty, K.T., Ilett, K.F., Davis, T.M., Safety and therapeutic efficacy of artesunate suppositories for treatment of malaria in children in Papua New Guinea. Pediatr. Infect. Dis. J. 2003; 22: 251–256.
[7] Mturi, N., Musumba, C.O., Wamola, B.M., Ogutu, B.R., Newton, and C.R., Cerebral malaria: optimizing management. CNS Drugs. 2003; 17: 153– 165.
[8] Turker, S., Onur, E., Ozur, Y., Nasal route and drug delivery systems. Pharm. World Sci. 2004; 26: 137–142.
[9] Vyas SP and Khar RK. Targeted and Controlled Drug Delivery, Novel Carrier System In: Vyas SP, Khar RK (eds): Submicron emulsion. 1st ed. CBS Publishersand Distributors, New Delhi. 2002; 295
[10] Moulik SP and Paul BK. Structure, dynamics and transport properties of micro emulsions. Advances in Colloid and Interface Science. 1998; 78: 99-195.

[11] Kumar Mukesh, Misra A, and Pathak K. Formulation and characterization of nanoemulsion of olanzepine for intranasal delivery PDA J Pharm Sci Technol 2009; 63:501-511.
[12] Porecha S, Shah T, Jogani V, Naik S, Misra A, Microemulsion based intranasal delivery system for treatment of insomnia, Drug Delivery 2009;16(3): 128-134.
[13] Rita JM, Pradip KG, Manish LU, Rayasa SR. Thermo reversible mucoadhesive gel for nasal delivery of sumatriptan. AAPS. Pharm Sci Tech. 2006; 7: E1–7.
[14] Mahajan HS, Gattani SG. In situ gels of Metoclopramide Hydrochloride for intranasal delivery: In vitro evaluation and in vivo pharmacokinetic study in rabbits. Drug Delivery 2010; 17(1): 19–27.