Formulation of quinine suppository for initiation of early treatment of malaria – a preliminary study

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Abstract

Background. In the management of malaria, there is the need for early initiation of treatment. An antimalarial drug for home use must be easy to administer, safe, effective and affordable. Parenteral quinine is the gold standard for treatment of severe malaria. A rectal formulation of quinine will therefore serve the purpose of early initiation of care in patients that lack easy access to medical centers. The main objective of this preliminary work was to develop a quinine suppository with adequate release properties that also meets the dual conditions of affordability and ease of administration.
Materials and Methods. Cocoa butter and Fattibase™ were used in the preparation of suppositories containing 200 mg quinine bisulphate. The release profiles of formulations with varying concentrations of polysorbate 80 (0 – 5%) were evaluated by in vitro dissolution in pH 8 buffer medium.
Results. The addition of polysorbate 80 improved the release of quinine significantly at 2 and 5%. Cocoa butter suppository with 1% polysorbate 80 released 73.6 mg quinine bisulphate in 1 hr while release from suppositories with 2% and 5% surfactant was higher. Fattibase™ suppositories had better release profiles than cocoa butter formulations. The formulation with 5% polysorbate 80 released 170 mg quinine in 1 hr. Formulations with the two bases released quinine in adequate quantities for the management of malaria.
Conclusions. The particle size of quinine is an important factor affecting the physical appearance and drug release from the suppository. The Fattibase™ suppositories were more stable but cost five times the price of the cocoa butter formulations. The cocoa butter formulations, however, still released quinine in sufficient quantities for the management of malaria. Cocoa butter formulations will be more affordable in resource-limited malaria-endemic regions of the world.

1 Introduction

The rectal route has been found to offer the same absorption possibilities as the oral, but the influence of the formulation is of critical importance [1,2]. In most cases the rate of absorption through the rectal route increases with increasing solubility of drugs in the vehicles and adjuvants [3,4]. Membrane-active adjuvants generally promote absorption more effectively in the colorectal region than in the upper gastrointestinal tract [5]. A suppository formulation of chloroquine phosphate in a mixture of polyethylene glycol with 0.5% polysorbate 80 produced adequate blood levels of chloroquine in children and would be therapeutic in the management of malaria and rheumatoid disease [6,7]. Pharmacokinetic trials of artemisinin, artemether and artesunate in the suppository form [8-10] indicated that the formulations were effective as rectal suppositories with 30-50% bioavailability. Intra-rectal artesunate may also be a useful alternative to parenteraly-administered artesunate in the management of moderate childhood malaria. The intra-rectal formulation was more rapidly absorbed in low doses than in high doses and parasite clearance kinetics was comparable with intravenous administration [11]. It may however be necessary to double or triple the quantity in the oral formulation in order to obtain a comparative therapeutic effect [11,12].

Various forms of intra-rectal quinine have produced encouraging bioavailability in children with falciparum malaria [12-14]. Compared to the intramuscular route, Quinimax® intrarectal solution, applied through an enema pump gave 40% bioavailability. Residual blood quinine concentration at 48 hrs was similar, so was mortality and temperature clearance amongst the trial groups. A cream formulation of quinine, prepared as an improvement on the enema had an encouraging efficacy profile with very good overall tolerance [15]. The formulations produced no major and/or irreversible complications. Early rejection, intestinal transit problems, and watery stools were the most common problems. In contrast, intramuscular Quinimax® led to residual pain, local inflammation, abscess and could produce lower extremity disability.

Two sustained release quinine rectal gels exhibited practically similar efficiency of dissolution (ED%) [16] and produced no damage on the rectal mucosa of rabbits. Another intra-rectal formulation, a sustained release of

Table 1. Composition, weight variation, hardness, and quinine content of the prepared Suppositories

Formulation	Composition				Mean weight variation (%) ±SD (n=20)	Hardness (month 0)	% Quinine content
	Cocoa butter (mg)	Fattibase™ (mg)	Quinine bisulphate (mg)	Polysorbate 80 (%)			
A	0.8570	-	200	0	-2.40±0.44	600	90.5
B	0.847	-	200	1	-2.60±0.42	800	93.2
C	0.8373	-	200	2	-1.00±0.23	1000	96.5
D	0.8077	-	200	5	-2.40±0.38	1200	97.8
E	-	0. 8776	200	0	-1.50±0.30	600	95.4
F	-	0.8699	200	1	-1.80±0.22	600	97.6
G	-	0.8625	200	2	-2.20±0.34	800	97.8
H	-	0.8393	200	5	-2.20±0.32	800	99.0

"transferring-conjugated solid-lipid-nanoparticles" (SLN) was able to deliver quinine dihydrochloride to the mouse brain [17].

Quinine intravenous injections are still widely used for the management of severe malaria in resource-limited regions of Nigeria [18]. A stable quinine suppository formulation in cocoa butter base tested on 12 healthy subjects had a poor release profile [19]. This present work seeks to improve on the previously studied intra-rectal quinine formulations with the development of an affordable, easy to administer quinine suppository formulation with an enhanced release profile. The work is a preliminary study with the main objective of developing a stable suppository formulation using a locally available base. The stability and in-vitro release of quinine from this formulation will be compared with another suppository made from a synthetic base with similar chemical composition with cocoa butter. This informed the choice of cocoa butter and Fattibase™.

Access to medical facilities is also a major concern in these regions. The work therefore has the secondary objective of developing an affordable formulation that can be used for the initiation of treatment of acute and complicated malaria in children by medical personnel and care givers. Cocoa butter is available locally and will therefore provide an affordable vehicle for such a formulation. A stable and affordable quinine suppository may contribute immensely to the reduction of child mortality in the poor districts of malaria-endemic regions [20].

2 Materials and Methods

The following materials were used to prepare the formulations: Cocoa butter or Theobroma Oil (Cocoa Industries Ltd, Ondo, Nigeria) is an oleaginous base that softens at 30ºC and melts at 34ºC and may require refrigeration under tropical conditions. Fattibase™ (Paddocks Laboratories, Minneapolis, USA) is a preblended suppository base that offers the advantages of a cocoa butter base with few of its drawbacks. It is composed of triglycerides derived from palm, palm kernel and coconut oils with self-emulsifying glyceryl monostearate and polyoxyl stearate used as emulsifying and suspending agents. The base is solid with a melting point of 35-37ºC, is stable with a low irritation profile and needs no special storage conditions. It is uniform in composition and has a bland taste with controlled melting range. It exhibits excellent mold release characteristics and does not require mold lubrication. In addition, we used quinine biphosphate (BDH, UK) and polysorbate 80 (Raymond Lab Chemicals, (UK), a surface-active agent.

2.1 Characterisation of bases and assays

The purity of the two suppository bases was ascertained by measuring the acid value, iodine value, saponification value, and melting range as specified in the BP. Assays were carried out on quinine bisulphate powder and the membrane-active agent polysorbate 80. All test readings were carried out in duplicate and mean values recorded.

2.2 Determination of displacement value

This is the number of parts by weight of the medicament that displaces one part by weight of the base. This value guides in making provision for density of added materials in a suppository formulation. Displacement values of quinine bisulphate and polysorbate 80 in cocoa butter and Fattibase™ were determined in the suppository mould.

2.3 Effect of particle size

The crystalline quinine bisulphate was passed through sieves of various sizes to evaluate the effect of particle

Table 2. Characteristics and displacement values of the two bases used in the study

	Characteristics				Displacement values	
Base	Iodine value	Acid value	Saponification value	Melting range ^{o}C	Quinine bisulphate	Polysorbate 80
Cocoa butter	38.75	4.3	189.34	34.2 – 35.7	1.39	1.01
Fattibase™	40.80	4.0	187.45	36.7 – 38.5	1.63	1.30

size on the appearance and release of active ingredient from the suppository formulations.

2.4 Preparation of suppositories

All suppositories were prepared by fusion method using a 1g stainless steel mould with 12 cavities. The moulds had been previously calibrated with cocoa butter. Four formulations were prepared with each of the bases (cocoa butter or Fattibase™) with the addition of 0, 1, 2 and 5% polysorbate 80. The eight resulting formulations are shown in Table 1.

2.5 Characterisation of suppositories

The formulated quinine suppositories were evaluated as prescribed in the British Pharmacopoeia (BP) 2010:

Appearance. The suppositories were physically examined for uniformity of mix and mottling;
Weight variation. Twenty suppositories were weighed individually; the mean and deviation from the mean of each suppository weight were calculated. This was repeated five times and standard deviations were calculated;
Melting range. This was evaluated using a modified USP method – A dissolution tester USP (Electrolab TDT-08L was filled with 900 ml of distilled water which was then heated to 30ºC. The suppository was introduced into the medium while heating continued until commencement of melting. The melting range was recorded (temperature at which melting commenced and temperature at which suppository was completely melted). Tests were carried out in duplicate;
Hardness. The hardness of the suppositories was evaluated using an Erweka suppository hardness tester (Type SBTE, NO 51249) operated at ambient temperature. One suppository was placed in the sample holder while incremental weights of 200g were added to the restraining bar and the weight that finally crushed the suppository was noted;
Chemical assay. The amount of quinine bisulphate in the formulations was determined by the modified assay method for quinine bisulphate (BP 2010).

2.6 Release profile

The ability of the formulations to release the active ingredient when administered was evaluated using the dissolution test method. The release profile of quinine bisulphate from the suppository was measured in a dissolution rate apparatus (USP, Electrolab TDT-08L) at various time intervals over a period of 1 hr in a pH 8 buffer medium. The temperature of the dissolution medium was pre-set at 37ºC. The absorbance of the active ingredient was measured with an Ultra Violet spectrophotometer at 281 nm. The results were subjected to test of variance at the statistical threshold of 95%.

2.7 Stability studies

Based on results of dissolution studies, short-term stability studies were carried out on the most promising formulation. Formulation H (Fattibase + 5% polysorbate 80) was placed on stability studies at ambient temperature (25ºC) and 4ºC in the refrigerator. The dissolution profiles, physical appearance and hardness were evaluated every month for a period of 3 months.

3 Results

3.1 Characterisation of bases

Table 2 shows the characteristics of the two bases. The iodine, acid and saponification values as well as the melting points for cocoa butter and Fattibase™ were within acceptable limits of BP, confirming the purity of the two bases.

3.2 Displacement value

The displacement values for quinine bisulphate and polysorbate 80 in cocoa butter and Fattibase™ were calculated and the results are shown in Table 2. The values are within the normal acceptable range and were taken into consideration in the formulation.

3.3 Assay of Quinine bisulphate and Polysorbate 80

Results of assays of the raw material, quinine bisulphate powder and Polysorbate 80 yielded a mean of 99.7 ±1% and 98.80 ± 1 % respectively. These values are within acceptable range (95-105%) of the BP.

3.4 Characterisation of suppositories

Appearance. The cocoa butter suppositories had a light yellow color which was uniformly distributed while the Fattibase™ suppositories were slightly off-white in color with a more uniform appearance. The quinine with particle size of 5micron was uniformly dispersed within the two bases with no mottling whatsoever. This was however not the case with the higher particle sizes of 10 and 15 micron. The particles were not uniformly distributed and resulting in mottled suppositories.

Weight variation. The BP (2010) states that individual weights of suppositories can vary by + 5% from the average weight of 20 suppositories. All the suppositories in the eight formulations were within the acceptable limits of + 5% variation from mean (Table 1).

Chemical assay. Assays of quinine in Cocoa butter suppositories ranged from 90.5% to 97.8%; while the Fattibase™ suppositories had quinine content of 95.4 – 99.0% (Table 1). Formulations A and B did not contain up to 95% active ingredient.

Melting Range of suppositories. Formulations A – D melted within the range of 30.5 – 34.5ºC, while Formulations E – H melted within 35.0 – 37.5o C.

Hardness test. The hardness of the cocoa butter suppositories increased with polysorbate 80 content while this trend was absent in the Fattibase™ formulations (Table 1). Hardness values for formulation H at 1, 2, and 3 months was 800, 1000, and 1000, respectively.

3.5 Release profile

Of the cocoa butter formulations, formulation A released 36.5% of the quinine content after 5 min in the dissolution medium, rising to 36.86% after 1 hr (Fig. 1A). Addition of 1% polysorbate 80 into the formulation (B) improved the release rate slightly with the release of 37.6 % (75.20 mg) quinine bisulphate after 1 hr. Release from formulation B increased consistently over time. The release profiles for formulations C and D (2% and 5% polysorbate 80) were slightly higher than for A and B but were erratic. Of the Fattibase™ formulations, formulation H, (Fattibase™ + 5% polysorbate 80) released the highest quantity of quinine bisulphate (85%, 170 mg) within the 1-hr study period (Fig. 1B).

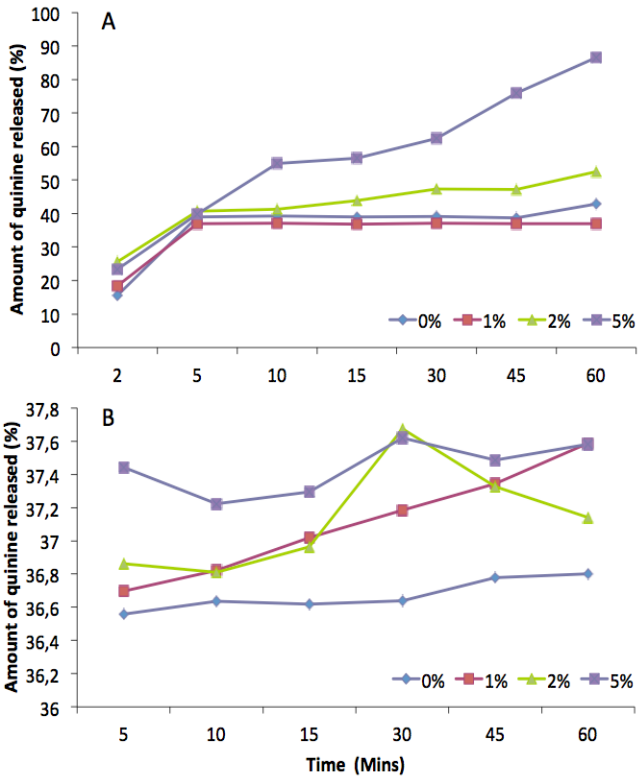

Figure 1. Amount of quinine released from cocoa butter (A) or Fattibase™ (B) suppositories augmented with varying levels of polysorbate 80 (0, 1, 2 or 5%).

The eight formulations from the two bases (A – H) satisfied the physical and chemical specifications for good suppository formulations. Weight uniformity and content uniformity were within specifications (Table 1). This is an indication that the two bases gave reproducible results. Suppositories from Fattibase™ were, however, smoother and with a better physical appearance. The hardness of the suppositories increased with surfactant concentration; the increase was more pronounced with the cocoa butter formulations (Table 1).

3.6 Stability studies

The results show consistent release profile rates throughout the 3 months of study for refrigerated Fattibase™ samples, keeping them stable over that period (Fig. 2A). Samples stored at ambient temperature (25o C) were not as stable (Fig. 2B). At the end of one month, the release of quinine from ambient samples was delayed, rising gradually from 30 min and attaining 80% release by 1 hr. At two months, the same samples released only 45% after 1 hr min while the 3-month old sample released only 40% steadily over the entire study period (1 hr). By the third

month, the samples stored at ambient temperature were showing signs of physical deterioration with brown mottling and mould growth.

The Fattibase™ suppository with 5% polysorbate 80 has good stability in the refrigerator. There was no statistical difference in the release profile over the three-month period (1 month P-value = 0.43; 2 month P-value= 0.28; 3 month P-value= 0.48). At the end of one month, the concentration of quinine released from the refrigerated sample was higher than the concentration released from samples stored at ambient temperature (between the time periods of 10–45 min) although the released concentration evened out after 1 hr. The concentration released from both samples was not statistically different (P=0.3). However, there was a slight significant difference between the percentage released in one month compared to that released after two months (P= 0.05). The same pattern was observed for two and three-month old samples (P= 0.05).

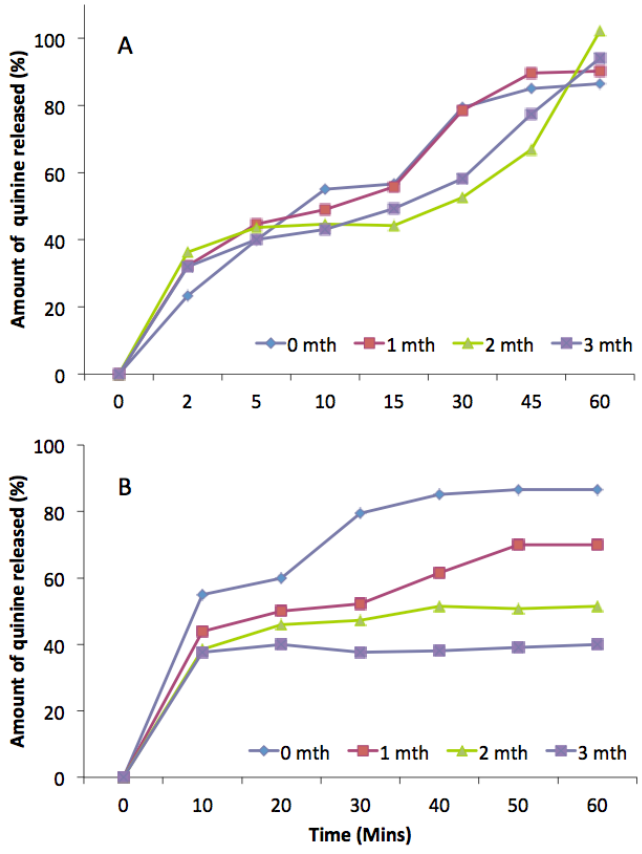

Figure 2. Release profile of quinine in Fattibase™ + 5% polysorbate 80 from refrigerated samples (A) or samples kept at ambient temperatures (B).

4 Discussion

Water solubility is the fundamental factor influencing the release rate of a drug substance from suppositories, either in lipophilic or hydrophilic excipients [21]. A drug with high water solubility quickly leaves the lipophilic excipient, producing a high concentration in the intra-rectal phase, which supports a high diffusion rate across the barrier. Quinine bisulphate salt is relatively water-soluble and the solubility increases with heating. It is therefore expected to have good release profiles from lipophilic bases such as Fattibase™ and cocoa butter. Surfactants are added to suppositories for the improvement of wetting properties. These membrane-active adjuvants generally promote absorption more effectively in the colorectal region than in the upper gastrointestinal tract [3]. Surfactants exhibit various degrees of wetting and they may even be the most important adjuvant influencing release rate [22].

The in vitro release profile and availability in the plasma has been correlated for many drug formulations [23,24]. A linear correlation between absorbed drug and dissolved drug may be obtained if a drug is absorbed completely after dissolution. Formulation A released 36.50% of the quinine content after 5 min in the dissolution medium, increasing to 36.86 % after 1 hr (Fig. 1); 73.6 mg of quinine bisulphate would therefore have been released in 1 hr, a quantity that would provide a therapeutic dose if completely absorbed in a child with 5 kg bodyweight (at a targeted dose of 10 mg/kg). A slight improvement was observed in drug release from Formulation B, 75.20 mg quinine bisulphate was released in one hour. The release profiles for Formulations C and D were erratic and the total amount released after 1 hr was only slightly higher than the concentration released from Formulation B (1% polysorbate 80). With an independent t-test, the effect of surfactant concentration on release from formulation with 0% and 1% polysorbate 80, was statistically significant (P=0.005). The mean variance between Formulation B and C (1% and 2% polysorbate 80) as well as Formulation C and D were also statistically significant. (P=0.03 and P=0.05, respectively). The concentration of surfactant significantly affected release of quinine bisulphate from the cocoa butter base.

From the descriptive data, the Fattibase™ suppositories (Formulations E – H) had better release profiles than the cocoa butter based formulations (A – D). Fattibase™ has as part of its components, the self-emulsifying glyceryl monostearate and suspending agent, polyoxyl stearate. The presence of these emulsifying agents in the base further enhances the release properties. Formulation E released 42%, (84mg) in 1 hr while Formulation F and G released 40% and 52% respectively (Fig 1B). An addition of 5% polysorbate 80 (Formulation H) increased the dissolution in 1 hr to 86.5% (173 mg). In the management of cerebral malaria, a 10 kg child (1 yr) will receive an oral

dose of 100 mg quinine. Unpaired t-tests showed no statistical difference between the quantity of quinine released from Formulations E and F (P= 0.33). However, the release from Formulations F and G were statistically different (P= 0.034). A statistical difference was also observed in the release profiles for Formulation G and H (P= 0.05). The effect of the surfactant, polysorbate 80 became significant at concentrations of 2% and 5%.

All the formulations released therapeutic quantities of quinine in children (10 mg/kg body weight) though the Fattibase™ suppositories had significantly higher release profiles. These formulations were also stable under refrigerated conditions. An increase in the dose of quinine in the cocoa butter formulation or a reformulation with other surfactant combinations may further improve release to achieve optimal therapeutic concentrations. Cocoa butter is cheap and locally available and will therefore be preferred for the local manufacture of the suppository in resource-limited malarious areas.

The Fattibase™ suppository with 5% polysorbate 80 has good stability in the refrigerator. The samples had a constant release rate throughout the 3 months storage period. The Repeated Measure ANOVA Procedure was used to examine the stability of the release profile of quinine in Fattibase™ + 5% polysorbate 80 stored in a refrigerator for four different time periods (month 0, 1, 2 and 3). There was no significant difference in the release prolife across months indicating stability over the course of study. The formulation stored at ambient temperature (20-25ºC) also had a steady and consistent release at one month and the suppositories were also physically stable. The mould growth observed by two months may have been due to contamination since the formulations did not contain preservatives and were not packed under sterile conditions.

From the melting range it was found that the FattiBase™ suppository will be stable under tropical conditions and should not require special storage conditions. The cocoa butter suppository will require refrigeration since the presence of polymorphs in the cocoa Butter could bring the melting point to as low as 18 ºC, a temperature that is below the ambient temperature of 20-25ºC. The three formulations from this study, i.e. cocoa butter + 1% suppository, FattiBase™ + 2% suppository and FattiBase™ +5% suppository all demonstrated good release profiles and, by extrapolation, have potential for adequate plasma concentration. These formulations can be further examined in clinical studies on healthy subjects to confirm efficacy and bioavailability.

Suppositories are relatively easy to administer to children with cerebral malaria. This accounts for the interest in this dosage form [20]. A stable and affordable quinine suppository may contribute immensely to the reduction of child mortality in Nigeria and other malaria-endemic countries especially within the rural and poor districts where access to medical care is low or absent [26]. Treatment can be commenced at home since the drug can be administered by caregivers.

5 Conclusions

Cocoa butter and Fattibase™ produced suppositories with satisfactory physico-chemical parameters. The formulations of suppositories in these two bases released quinine in a pH 8 buffer medium. The release of the quinine was improved with the addition of polysorbate 80 as a release enhancer. The best release profile was obtained with quinine in FattiBase™ + 5% polysorbate 80 with the release of 86.5% (173 mg) of the quinine content. The concentration of quinine released from cocoa butter + 1% polysorbate 80 (75.20 mg) and FattiBase™ + 2% polysorbate 80 (104 mg) will also be adequate for the management of malaria. The formulation of Fattibase™ was stable for one month at ambient temperature (25-30ºC) and 3 months in the refrigerator. It can therefore be safely stored in homes for one month without adverse effect on drug content, release and physical appearance of the suppository. The cocoa butter suppository will require refrigeration since the presence of polymorphs in it can bring the melting point below the ambient temperature of 25-30ºC.

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