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Comparative in vitro–in vivo study of two quinine rectal gel formulations

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Abstract

The main objective of this work was to develop and evaluate rectal quinine paediatric formulations to treat acute uncomplicated malaria attack in some African countries. Developed dosage forms must be able to assure a prolonged release in the rectum but not too much so as to avoid product expulsion by the child anus. Two quinine rectal gels, namely mucoadhesive (MA) gel and thermosensitive (TS) gel, containing 20 mg quinine base/g were developed and evaluated in vitro and in vivo in the rabbit. The MA and the TS gels contained hydroxypropyl methylcellulose 4000 (HPMC) and poloxamer 407, respectively. The calculated in vitro release exponent (n) values suggested that drug was released from both gels by non-Fickian diffusion. Both gels exhibit practically similar efficient of dissolution (ED%) which was not reflected in the plasma and, therefore, quinine bioavailability from MA gel was found to be higher than that obtained from TS gel and their $AUC_{0-\infty}$ were statistically different ($P = 0.0006$). The $t_{1/2}$ values of quinine were significantly higher for Hydrogels than for IV and rectal solutions. MRT values displayed by TS gel and MA gel were not statistically different but were about 3.8- and 1.3-fold, respectively, larger than those obtained for IV solution and rectal solution, respectively. These results confirm the sustained-release behaviour of both hydrogels in the rabbit. Tolerability study of hydrogels didn't show any damage on the rectal mucosa of the rabbit.

Keywords: Bioavailability; Hydroxypropyl methylcellulose; Malaria; Paediatric; Poloxamer; Quinine pharmacokinetics; Rectal gels

1. Introduction

In sub-Saharan Africa, malaria mortality based on investigations conducted in rural areas (Gambia, Kenya, R.D. Congo, and Burkina Faso) has been evaluated around 10/1000 in <1-year-old and 6/1000 in 1–4-year-old children (Gazin de Raucourt, 1990; Greenwood et al., 1987). Cerebral malaria, prevailing in low malaria transmission areas and anaemia in high transmission areas represent the main cause of severe malaria in children, who are not able to take any per os medication at this stage because of unconsciousness or nauseas (Brewster et al., 1990; Bruce-Chwatt, 1952; Molyneux, 1995; Warrell et al., 1990). Thus, the less expensive and recommanded regimens by WHO are based on quinine administered intravenously or intramuscularly (Mansor et al., 1990; Pasvol et al., 1993; Warrell et al., 1990). However, when

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administered in poor hygiene conditions, such injections may cause infection (HIV, tetanus, hepatitis), or paralysis (Barennes, 1999; Yen et al., 1994). Perfusions are not often available and the child must be conducted in a health center, and many children die during transportation because of the treatment delay (White and Krishna, 1989). Moreover, among children admitted to a hospital, 50–70% die the first hours following their admission whatever the administered treatment (White and Krishna, 1989). These observations indicate that there is a need to improve an early and easy treatment in children, which can be administered in house by educated mothers or in a rural dispensary by a health technician (Barennes et al., 1998; English et al., 1996; Murphy et al., 1997; White, 1995).

Rectal route has been chosen because it is easy to administer in children that are unable to swallow any drug. These last years, Barennes et al. (1995, 1996a, 1999) have reported that Quinimax® (a water-soluble combination of cinchona alkaloids) appeared to be efficacious and well tolerated by rectal route in the treatment of acute uncomplicated malaria of the child. However, bioavailability was hampered by loss of part of the liquid solution during or shortly after rectal introduction, and some inflammatory effects were reported. Barennes et al. (1996b) have also tested a rectal cream formulation of quinine gluconate. It was found that absolute bioavailability of quinine in the tested intrarectal cream was 36% whereas its relative bioavailability versus intramuscular solution was 51%. This low measure of bioavailability may be caused by the difficulty in controlling dosage administration. The authors have noted the good tolerability of this cream. Therefore, in general, none of the reported quinine rectal formulations had at once a good tolerability and a high bioavailability level. However, the development of the above reported quinine rectal formulations have to deal with the implementation of efficiency ratio between tolerance, individual acceptability, and bioavailability.

The use of conventional solid suppositories is often associated with many limitant problems (absorption irregularity, patient discomfort and acceptability, etc.). Recently, liquid suppositories have been proposed as alternatives to conventional suppositories for the administration of various drugs by rectal route (Choi et al., 1998a; Choi et al., 1998b; Kim et al., 1998; Miyazaki et al., 1995; Ryu et al., 1999; Watanabe et al., 1993; Yun et al., 1999). The proposed liquid suppositories are hydrogels which exist as liquid in vitro but gel in vivo.

The aim of this work was to develop paediatric rectal quinine hydrogels. These formulations are dedicated to be used in the treatment of acute malaria attack or in uncomplicated cases with high digestive tract involvement as an alternative to parenteral administration of quinine. In this study, two quinine intrarectal formulations (temperature-sensitive “TS” and mucoadhesive ‘MA” gels) were developed in order to improve quinine rectal bioavailability and tolerability. Evaluation of the developed gels was carried out at both in vitro and in vivo levels.

2. Materials and methods

2.1. Materials

Quinine hydrochloride, lactic acid and propylene glycol were obtained from Sigma (St. Louis, MO). The gel-forming polymers were Methocel® 4000 mPa s (hydroxypropyl methylcellulose “HPMC”) and Lutrol® F127 (poloxamer 407) supplied, respectively, by Colorcon (Dartford Kent, UK) and BASF (Lyon, France). Dialysis membrane (Spectra/Pore®, MC CO 12000-14000, 16 mm diameter) was obtained from Spectrum Laboratories (Rancho Dominguez, CA, USA). All other components and solvents were of analytical grade and purchased from Sigma.

2.2. Preparation of quinine solutions and gels

All quinine formulations (solutions and gels) were prepared using quinine hydrochloride at the theoretical concentration of 24 mg/g (equivalent to 20 mg of quinine base/g). All quinine concentrations given below in this paper will be expressed as quinine base. pH values of rectal preparations were adjusted close to 5.3 to avoid quinine precipitation.

2.2.1. Intravenous solution

This solution was prepared just before administration. 2.4 g of quinine hydrochloride (equivalent to 2 g of quinine base) was dissolved in sterile distilled water (100 ml). After quinine hydrochloride was completely

dissolved, the solution was filtered through a membrane filter (pore size 0.22 μm). The pH value of this solution was 6.3.

2.2.2. Rectal solution

The 0.5% lactic acid aqueous solution (7 g) was added to deionised water (70.6 g). The 2.4 g of quinine hydrochloride (equivalent to 2 g of quinine base) was then dissolved in the liquid mix at room temperature under magnetic stirring until clear solution was obtained. Propylene glycol (20 g) was finally added to the solution.

2.2.3. Rectal mucoadhesive gel

Propylene glycol (20 g) was mixed with 0.5% lactic acid solution (7 g) and deionised water (69.1 g). The 2.4 g of quinine hydrochloride (equivalent to 2 g of quinine base) was then dissolved in the liquid mix at room temperature and HPMC (1.5 g) was slowly added under moderate stirring (250 rpm) using an electro-mechanical stirrer (Heidolph Instruments, RZR 2051, Schwabach, Germany) until the obtaining of clear gel.

2.2.4. Rectal temperature-sensitive gel

Propylene glycol (20 g), 0.5% lactic acid solution (7 g) and quinine hydrochloride (2.4 g equivalent to 2 g of quinine base) were added to deionised water (51.6 g) at room temperature. When solution became clear, it was cooled down to 4 °C. Lutrol® F 127 (18 g) was then slowly dispersed in the solution under continuous stirring (1000 rpm) using an electro-mechanical stirrer. The dispersion was finally left at 4 °C until a clear solution was obtained.

2.3. Measurement of gelation temperature of the thermosensitive gel

The gelation temperature was measured according to the method reported by Yun et al. (1999). Briefly, 10 g of each gel was placed in a transparent glass vial with a magnetic bar (15 mm × 6 mm). The preparation was heated, from 20 °C, at an increased temperature of 1 °C/min with a constant stirring of 100 rpm. The temperature at which the magnetic bar stopped moving was taken as the gelation temperature. The same experiment was carried out in similar conditions without magnetic bar but with monitoring the viscosity of the gel between 20 and 37 °C.

2.4. Measurement of viscosity

Viscosity of hydrogels was measured in a cone viscometer (Model DV-3, Brookfield, USA). A 50 g sample of gel was placed in a 100 ml beaker. At first, viscosity measurement was recorded at 20 °C and then temperature was raised to 37 °C by a water jacket through which water was circulated at 37 °C from a thermostat bath. Each measurement was repeated three times and mean value was calculated.

2.5. In vitro drug release kinetics from rectal formulations

In vitro release of quinine from rectal formulations was monitored by the USP basket method at a rotating speed of 100 rpm in 1000 ml deionised water at 37 ± 0.5 °C. An automatic dissolution-rate system (Sotax AT 7, Switzerland) for continuous spectrophotometric analysis was used. Two grams of each formulation was introduced in a Spectra/pore® dialysis tubing after being tied tightly one end. Then, the other end was sealed to obtain a bag which was immediately put into a basket. Six bags were prepared for each formulation. Absorbance was measured at 235 nm during 6 h. The rectal solution of quinine hydrochloride was used as reference.

The dissolution time of 50% (DT50) and the dissolution time of 80% (DT80) of the initial drug content of each rectal formulation were extracted from the dissolution profiles. Dissolution efficiency was calculated over 6h ($ED_{6\,h}$%) (Khan, 1975; Khan and Rhodes, 1972).

2.6. Pharmacokinetic study

2.6.1. Animals

Experiments were carried out on male albinos rabbits (Elevage de la Faurie, Cubjac, France) weighing 2–2.5 kg. The animals were fasted for 24 h prior to and 10 h following treatment but had free access to tap water. They were randomly assigned in four groups (six per group). One group received quinine solution intravenously. The three other groups were given three distinct quinine rectal formulations (one formulation each group). All experiments on animals reported in this

paper were conducted according to the European Communities Commission Directive 87/302/EEC (EEC, 1987) and in conformance with the French Ministry of Agriculture Permission No. 03640.

2.6.2. Administration and blood samples collecting

Regardless of the type of dosage form, a single dose equivalent to 12 mg of quinine base per kg of body weight was given to each animal. Intravenous solution of quinine, containing the calculated dose in a final volume of 1.5 ml, was administered over 1 min through the marginal ear vein. The rectal formulations were administered quickly through a rectal cannula. Before the rectal administration, the rectum of the rabbit was emptied using a Foley cannula and after rectal administration, the rectum of the rabbit was clamped with a clip in order to prevent leakage of the product.

2.6.3. Determination of quinine in plasma

Following administration of various formulations containing quinine, blood samples (1 ml) were taken at defined times up to 10 h using heparinized tubes, by marginal ear vein puncture. Plasma was isolated by centrifugation (3000 rpm), frozen and stored at −25 °C until further processing.

Quinine levels in plasma were determined according to a modified HPLC method (Nielsen et al., 1994). The HPLC equipment consisted of a Model P200 pump and an AS100XR Autosampler (Thermo Separation Products, Les Ulis, France), a RF-535 fluorimetric detector (Shimadzu, Croissy Beaubourg, France) and a chromatography work-station Baseline 810 (Waters, Saint-Quentin en Yvelines, France). Separation was achieved at room temperature on a (250 mm × 4 mm) Hyperchrome® column packed with 5 μm Lichrospher 60 RP select B (Bischoff, Leonberg, Germany). The mobile phase was a mixture of acetonitrile, methanol and a sodium perchlorate pH 2.5 solution (14:16:70 (v/v)). The pH 2.5 sodium perchlorate solution was obtained by dissolving 14.05 g of sodium perchlorate and 1.6 ml of 60% perchloric acid in 5 l of water. The flow rate was 1.5 ml/min. Fluorimetric detection was performed at an excitation wavelength of 330 nm and emission wavelength of 430 nm.

2.6.4. Sample pre-treatment

Extraction of quinine was carried out according to the Nielsen et al. (1994) method. Briefly, 250 μl of plasma was mixed with 25 μl of 1 M NaOH. Quinine was then manually extracted with 3 ml methylene chloride. The mixture was centrifuged at 1500 rpm. After elimination of the aqueous phase, 2 ml of methylene chloride was transferred into glass tube and evaporated to dryness at 45 °C. The residue was then dissolved in 1 ml mobile phase. A 50 μl aliquot was injected onto the column. The last operation was carried out in triplicate.

2.6.5. Pharmacokinetics

C_{max} and T_{max} were determined from rectal data. The area under the curve ($AUC_{0-\infty}$) of quinine concentration in the plasma was calculated following the trapezoidal rule during the experimental period (AUC_{0-10}) with extrapolation to infinity according to equation:

$$AUC_{10-\infty} = \frac{C_{10}}{k_e}$$

C_{10} is the quinine plasma concentration at 10 h and k_e the terminal elimination rate constant. The total body clearance (Cl_T) and the apparent volume of distribution (V_d) were calculated as:

$$Cl_T = \frac{\text{dose} \times f}{AUC_{0-\infty}} \text{ and } V_d = \frac{Cl_T}{k_e}$$

f is the fraction of drug absorbed after rectal administration ($f = 1$ after intravenous administration).

The mean residence time (MRT) of quinine in the body was calculated by the formula ($AUMC_{0-\infty}$/$AUC_{0-\infty}$), where $AUMC_{0-\infty}$ represents the area under the moment curve from time 0 to infinity. The mean absorption time (MAT) after rectal administration was determined by making the difference between MRT of each rectal formulation and MRT after intravenous administration. The constant of absorption (k_a) was calculated as $k_a = 1/MAT$.

2.7. Tolerability study

This investigation was carried out firstly on all animals used in the pharmacokinetic study and to which quinine formulations were given by rectal route. Secondly, 4-fold increased doses (48 mg of quinine/kg) of MA gel, TS gel and rectal solution were given by rectal route, respectively, to three other groups of rabbits (one formulation each group, six per group).

For this investigation, the rabbits were previously prepared as it was described above in the pharmacokinetic study. All animals were sacrificed 6 h after dosing. Rectum and distal colon were excised, slit open and rinsed carefully with normal saline solution. The mucosal surface was then examined for the detection of superficial macroscopic lesions using a low-power stereo microscope.

2.8. Statistics

All data were expressed as mean value ± S.D. Statistical analysis was performed using ANOVA test (Scheffe test). Mean differences were considered statistically significant at a level $P < 0.05$.

3. Results and discussion

3.1. Characteristics of quinine formulations

All tested quinine formulations were clear at the laboratory temperature (22 °C). Quinine base content (20 mg/g) and pH values were chosen in order to obtain clear formulations in which quinine remained entirely dissolved even when they are stored at +4 °C or at the body temperature after rectal administration. Lactic acid was added in order to maintain pH value close to 5.3 and to avoid quinine precipitation. Propylene glycol was used essentially to modify the temperature of gelation of the TS gel and to modulate the release kinetics of quinine from both TS and MA gels. Preliminary experiments have allowed optimizing the choice of the amounts of propylene glycol and HPMC to be used (data not shown). Preliminary study was carried out on several polymers currently used in the formulation of gels namely poloxamers, carbopols, carboxymethylcellulose sodium (CMC sodium) and HPMC. Carbopols were discarded because quinine precipitated when medium was neutralized. At pH 5.3 and equal polymer concentrations, HPMC aqueous solutions were found to be more compatible with quinine hydrochloride and more viscous than CMC sodium solutions which exhibit maximum viscosity and stability at pH 7–9 (Rowe et al., 2003). The main characteristics of both MA and TS gels used in this study are shown in Table 1.

TS gel viscosity elevated quickly following the elevation of the temperature between 20 and 24 °C and then progressively until the gelation temperature was reached (31 ± 0.5 °C). At this temperature the magnetic bar was stopped as the TS gel was strongly gelled and looked like a jelly. As shown in Fig. 1, the viscosity of the TS gel continued to increase between 31 and 37 °C but more and more slowly. When 37 °C was reached, the viscosity of the TS gel was of 27,300 mPa s, whereas that of the MA gel was only of 1470 mPa s (Table 1).

3.2. In vitro kinetic drug release

Profiles of drug release from various quinine formulations are shown in Fig. 2 and their main dissolution characteristics are reported in Table 2. Drug release was generally retarded by the addition of polymers and there was no burst effect. The amount of released drug from both tested hydrogels increased quickly at the beginning of the test but the release kinetic from MA gel was faster. This finding was confirmed by the DT50 values (52 ± 2.3 and 72 ± 3.5 min) calculated for the MA and the TS gels, respectively. One hundred forty minutes later, the amount of released drug from both hydrogels was nearly the same (about 80%) and no significant difference was found between the DT80 values obtained from both hydrogels. Three hours after the beginning of the dissolution test, the release kinetic from all formulations became very slow. At this time, the cumulative amount of released drug was slightly but significantly larger ($P < 0.002$) from TS gel (87 ± 1.5%) than from

Table 1
Main characteristics of mucoadhesive and thermosensitive quinine rectal gels

Quinine formulations	pH at 22 °C	Viscosity at 20 °C (mPa s)	Viscosity at 37 °C (mPa s)	Temperature of gelation (°C)
Mucoadhesive gel	5.37	2825	1470	–
Thermosensitive gel	5.30	750	27300	31 ± 0.5

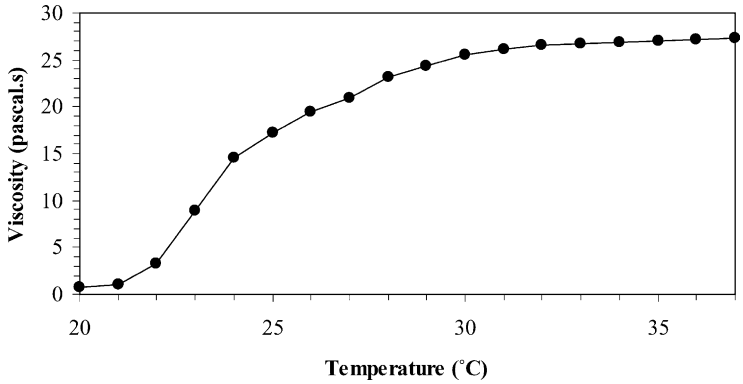

Fig. 1. Viscosity increase of the TS gel according to the temperature between 20 and 37 °C.

MA gel (85 ± 0.9%). The cumulative amount of released drug over the 6 h test was significantly higher from TS gel (95 ± 0.9%) than from MA gel (90 ± 0.4%). However, efficiency of dissolution (ED%) from both hydrogels was similar (74%). The acceleration of the drug release from the TS gel 2 h after the beginning of the test was likely due to the decrease of viscosity in the gel. Actually, at the end of the test, the visual inspection of the dialysis bags showed a less viscous content than expected but samples were too small to measure the viscosity of the gel.

To understand the release mechanisms of quinine from rectal gels, we attempted to describe the rate of release using the semi-empirical model of Korsmeyer et al. (1983) and more precisely the equation proposed

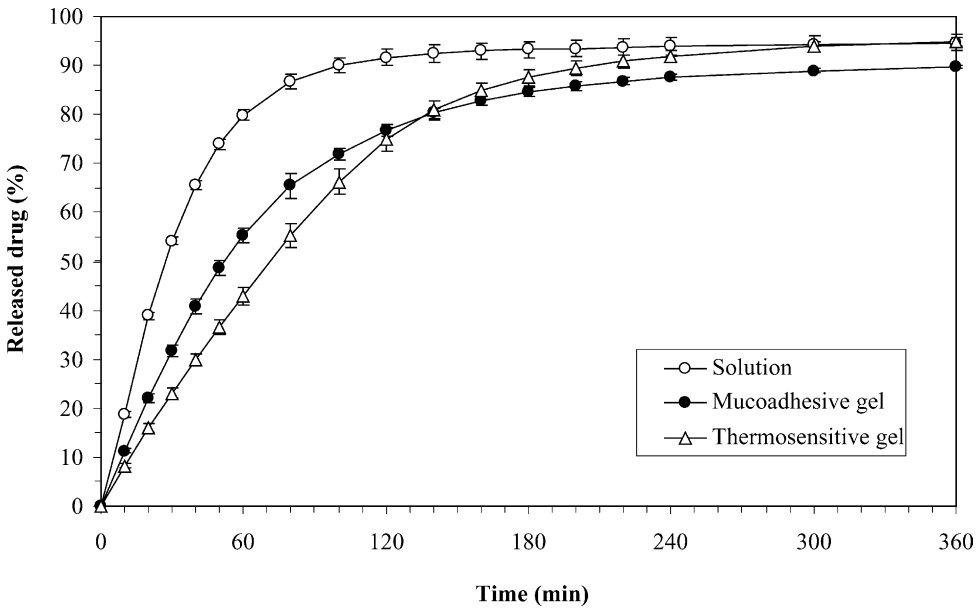

Fig. 2. Percentage of quinine released in deionised water (pH 6.9) versus time from rectal formulations (solution, MA gel and TS gel) (mean ± S.D., $n = 6$).

Table 2
Characteristics of the in vitro release (deionised water pH 6.9) of quinine from rectal formulations

Dissolution characteristics	Rectal solution (A)	Mucoadhesive gel (B)	Thermosensitive gel (C)	Statistics Scheffe-test
DT50 (min)	27 ± 0.5	52 ± 2.3	72 ± 3.5	ABC
DT80 (min)	61 ± 2.8	139 ± 7.3	137 ± 6.7	ACB
CARD 3 h (%)	93 ± 1.7	85 ± 0.9	87 ± 1.5	ACB
CARD 6 h (%)	95 ± 1.7	90 ± 0.4	95 ± 0.9	CAB
$ED_{(6\,h)}$ (%)	85 ± 1.4	74 ± 0.8	74 ± 1.3	ABC
Release exponent (n)	1.02	1.12	1.10	
Kinetic constant (a (%/h^n))	79.8492	55.2094	42.9843	
Coefficient of correlation (r)	0.8778	0.9509	0.9742	

DT50: dissolution time of 50% of the initial drug sample content, DT80: dissolution time of 80% of the initial drug sample content, $CARD_{3\,h}$: cumulative amount of released drug over the first 3 h; $CARD_{6\,h}$: cumulative amount of released drug over 6 h, $ED_{6\,h}$ (%): efficiency of dissolution calculated over 6 h. All these data are expressed as mean values ± S.D. n was determined using the portion of release curve where $M_t/M_\infty < 0.6$. In the statistics column, the various values are given in descending order magnitude. All underlined values are statistically not different ($P > 0.05$).

by Peppas (1985):

$$\frac{M_t}{M_\infty} = at^n$$

or the logarithmic form of this equation:

$$\log\left(\frac{M_t}{M_\infty}\right) = \log(a) + n\log(t)$$

where (M_t/M_∞) is the fraction of released drug at time (t), (a) a characteristic constant of the dosage form and (n) the release exponent, indicative of the drug release mechanism. The kinetic parameters (n and a) were calculated from the plot of $\log(M_t/M_\infty)$ versus $\log(t)$ (Fig. 3), where $(M_t/M_\infty) < 0.6$.

As shown in Table 2, all mean n values are higher than 0.5 suggesting that quinine was released from gels by non-Fickian diffusion. The a values indicate that quinine was released more slowly from gels than from solution and release was slower from the TS gel than from the MA gel. Therefore, TS gel, for which the highest viscosity (27,300 mPa s) was found at 37 °C, had the smallest a value (42.9843%/h^n). A Similar result has been already reported for acetaminophen in liquid suppositories (Choi et al., 1998a; Kim et al., 1998) and for insulin in liquid suppository (Yun et al., 1999) and were attributed to the high gel strength of the liquid suppository, which was defined by Yun et al. (1999) as the viscosity of liquid suppository at the physiological temperature. Moreover, 60% of the theoretical quinine content was released from the TS gel over 87 min following a kinetic close to zero order ($n = 1.1$ and $r = 0.9964$).

3.3. Pharmacokinetic study

Fig. 4 presents the mean plasma concentration versus time profiles of quinine after intravenous administration of quinine solution as well as after rectal administration of quinine solution and hydrogels.

The blood sampling over 10 h was adequate for the purposes of the study namely to assess the feasibility of using quinine hydrogels by rectal route and evaluate their bioavailability. However, to obtain more accurate values for t_{max}, blood sampling should have begun earlier than 0.25 h. In addition, it was necessary to clamp the rectum of the rabbits to prevent leakage of product, especially for the rectal solution, otherwise the loss of product was very significant.

The concentration–time profiles produced after intravenous administration of quinine hydrochloride (12 mg/kg) were characterized rather by a bi-exponential decline in most of the rabbits used in this study. Therefore, a bi-compartmental model was found to be more suitable for the description of the pharmacokinetic disposition of quinine in the rabbit. Hasan et al. (1990) had found that a one-compartmental model was adequate for the description of the pharmacokinetic behaviour of the quinine in the rabbit. However, the values of the pharmacokinetic parameters calculated according either to one-compartmental or to bi-compartmental model had nearly similar values.

The main pharmacokinetic parameters are summarized in Table 3. Among all rectal formulations, MA gel showed the highest C_{max} (698 ± 150 ng/ml) but

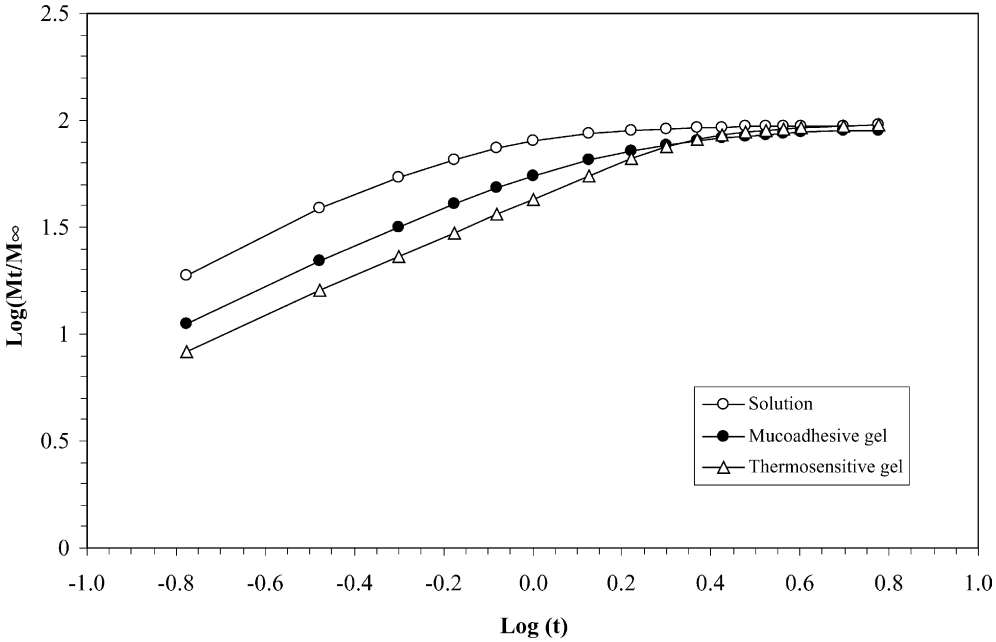

Fig. 3. The log–log plots of mean released fractions of quinine in deionised water (pH 6.9) against time from rectal formulations (solution, MA gel and TS gel).

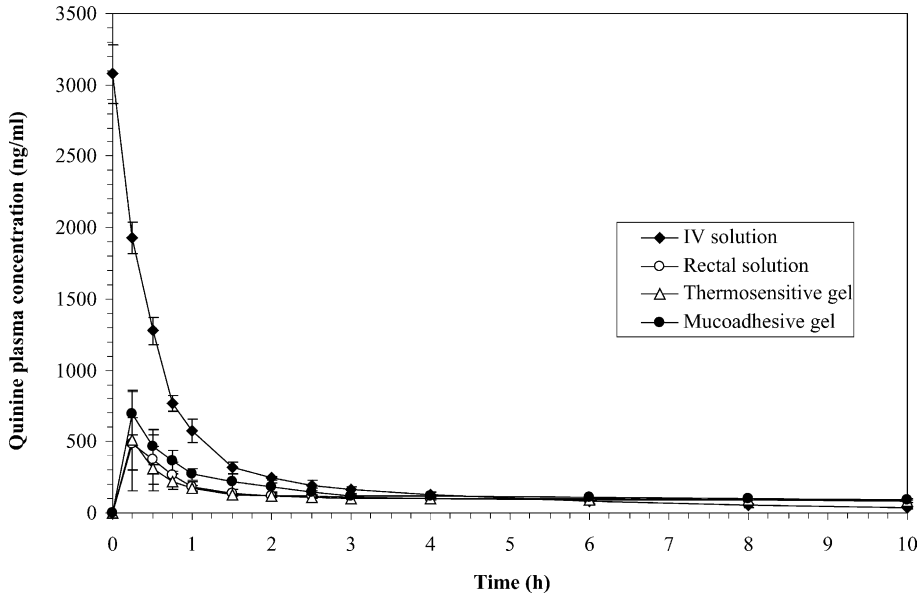

Fig. 4. Plasma concentration–time profiles of quinine after intravenous bolus administration and rectal administration of three quinine formulations (solution, MA gel and TS gel). (Mean ± S.D., $n = 6$).

Table 3
Bioavailability and pharmacokinetic parameters (mean values ± S.D.) following intravenous and rectal administration of quinine formulations (12 mg of quinine base/kg) to four groups of rabbits (one formulation each group)

Pharmacokinetic characteristics	Intravenous administration	Rectal administration		
		Solution	Thermosensitive gel	Mucoadhesive gel
C_{max} (ng/ml)	–	593 ± 181 (30)	509 ± 351 (69)	698 ± 150 (21)
t_{max} (h)	–	0.25 ± 0.0	0.25 ± 0.0	0.25 ± 0.0
AUC_{0-10} (ng h/ml)	2589 ± 86 (3)	1324 ± 167 (12)	1179 ± 151 (13)	1531 ± 157 (10)
$AUC_{0-\infty}$ (ng h/ml)	2711 ± 104 (4)	1787 ± 189 (10)	1798 ± 156 (9)	2340 ± 139 (6)
F (%)[a]	100	62.7	66.3	86.3
F' (%)[b]	–	100	105.8	137.7
k_e (h^{-1})	0.278 ± 0.027 (10)	0.198 ± 0.0228 (11)	0.134 ± 0.0104 (8)	0.119 ± 0.0155 (13)
$t_{1/2}$ (h)	2.52 ± 0.245 (10)	3.54 ± 0.40 (11)	5.19 ± 0.40 (8)	5.92 ± 0.68 (11)
MRT (h)	2.32 ± 0.294 (13)	6.77 ± 0.59 (9)	8.73 ± 0.95 (11)	8.87 ± 0.96 (11)
MAT (h)	–	4.71	6.40	6.55
k_a (h^{-1})	–	0.2121	0.1561	0.1527
Cl_T (l/(h kg))	4.43 ± 0.16 (4)	4.23 ± 0.46 (11)	4.45 ± 0.36 (8)	4.46 ± 0.29 (6)
V_d (l/kg)	16.08 ± 1.32 (8)	21.50 ± 2.33 (11)	33.43 ± 4.27 (13)	38.20 ± 5.55 (14)

C_{max}: maximum plasma concentration; t_{max}: time to reach C_{max}; F: asolute bioavailability; F': relative bioavailability; k_e: constant of elimination rate; $t_{1/2}$: terminal half-life; AUC_{0-10}: area under the plasma level curve between time 0 and 10 h; $AUC_{0-\infty}$: total area under the plasma level curve; MRT: mean residence time; MAT: mean absorption time; k_a: constant of absorption; Cl_T: total clearance; V_d: apparent volume of distribution; between brackets are indicated the intersubject coefficients of variation (CV%).

[a] IV solution was used as reference standard.

[b] Rectal solution was used as reference standard.

differences were not statistically significant probably because of the high inter-individual variability. The analysis of these results allowed verifying that the in vitro release kinetics were reflected in the plasma levels after rectal administration. Therefore, the highest C_{max} value in vivo was obtained for the MA gel, which had the faster drug release in vitro.

In comparison with the $AUC_{0-\infty}$ obtained for the rectal solution of quinine (1787 ± 189 ng h/ml), $AUC_{0-\infty}$ for the MA gel (2340 ± 139 ng h/ml) was significantly higher ($P < 0.0001$), whereas that calculated for the TS gel (1798 ± 156 ng h/ml) was nearly similar. The lower MRT and $t_{1/2}$ values found for the rectal solution (Table 3) as well as its possible leakage through the anuses of the rabbits could explain its low $AUC_{0-\infty}$.

AUC_{0-10} and $AUC_{0-\infty}$ for the MA gel were significantly higher ($P = 0.01$ and 0.0006, respectively) than those found for the TS gel. However, it must point out that this result was not in agreement with in vitro $ED_{6\,h}$%, which was nearly similar for both gels, suggesting that diffusion of drug from the TS gel was more rapid in vitro than in vivo. The higher $AUC_{0-\infty}$ found for the MA gel could be explained by a larger extent of its rectal absorption due to its relatively low viscosity at the physiological temperature as compared with that of the TS gel. Thus, the best bioavailability, either absolute bioavailability (F) or relative bioavailability (F'), was obtained from MA gel ($F = 86.3$ and 137.7%) (Table 3). However, the constant of absorption values (k_a) found for MA and TS gels (Table 3) indicated that the rate of absorption was nearly similar for both gels.

No significant difference was found between the mean $t_{1/2}$ values of quinine given to rabbits either in the MA gel (5.92 ± 0.68 h) or in the TS gel (5.19 ± 0.40 h). Moreover, for both rectal gels $t_{1/2}$ was significantly longer ($P < 0.0001$) than for rectal solution (3.54 ± 0.40 h). Thus, in comparison with intravenous solution, $t_{1/2}$ was 2.35- and 2.0-fold increased by the MA gel and the TS gel, respectively, whereas it was 1.7-fold increased by the rectal solution.

After intravenous administration of quinine hydrochloride solution, the mean $t_{1/2}$ value (2.52 ± 0.24 h) was 2-fold longer than that reported by Hasan et al. (1990) after intravenous administration of quinine hydrochloride to rabbit. In addition, the $t_{1/2}$ values found in the rabbit was shorter than those

reported in healthy human subjects either by Salako and Sowunmi (1992) (12.6 ± 2.5 h) or by Karbwang et al. (1993) (9.9 h) after IV infusion over 1 and 4 h, respectively. However, It was interesting to point out that $t_{1/2}$ of quinine from the rectal solution was close to that reported by Barennes et al. (1995) after rectal administration of Quinimax® to children with acute uncomplicated *Plasmodium falciparum* malaria.

After intravenous administration of quinine solution MRT found (2.32 ± 0.29 h) was significantly lower ($P < 0.0001$) than that found for rectal formulations (Table 3) but was close to that reported by Hasan et al. (1990) after IV administration to the rabbit. Moreover, MA and TS gels displayed a similar MRT (8.87 ± 0.96 and 8.73 ± 0.95 h, respectively) which was significantly higher ($P < 0.02$) than that found for the rectal solution suggesting their sustained-release behaviour in the rabbit.

No significant difference was found between the total plasma clearance Cl_T after intravenous administration (4.43 ± 0.16 l/(h kg)) and those obtained after rectal administration of quinine formulations. Since the Cl_T (Table 3) was not altered, it can be deduced that rectal gels did not affect the rate of the elimination processes. However, Cl_T after IV administration was 3.25-fold higher than that already reported in the rabbit (Hasan et al., 1990).

V_d for the MA and TS gels (Table 3) were significantly higher ($P < 0.0001$) than V_d found for the IV solution (16.08 ± 1.32 l/kg) and 1.8- and 1.6-fold, respectively, higher ($P < 0.003$) than V_d obtained for the rectal solution. Moreover, V_d for IV solution was close to that reported by Hasan et al. (1990). The increase in the $t_{1/2}$ and V_d of quinine after rectal administration of gels as compared to rectal solution would confirm the slow release of drug from both gels. This result is in agreement with in vitro dissolution profiles of both quinine rectal gels.

3.4. Tolerability study

Whatever the rectal quinine formulation used in the pharmacokinetic study, all the rabbits to which a single dose of quinine (12 mg/kg) was given did not show any kind of lesions on the rectal mucosa.

Probably, the administration of single dose to rabbits was insufficient to induce lesions. A multiple dosage regimen was not considered due to the difficulty of fasting the rabbits during many consecutive days.

Evidence of irritation, associated with ulceration and rectal bleeding, was observed in all rabbits to which quinine solution (48 mg/kg) was administered by rectal route. A very small irritation was shown on the rectal mucosa of the rabbits to which quinine hydrogels (48 mg/kg) were administered. A likely explanation for this is the presence of higher free quinine concentration on the contact of the rectal mucosa after administration of solution than after administration of hydrogels. The pH of the quinine hydrochloride solution (6.30) cannot be responsible for these adverse effects because only minor side effects were reported after rectal repeated dosing of Quinimax® (pH 4.55). In order to increase Quinimax® tolerability by rectal route, Barennes et al. (1999) recommended diluting it in a small volume of water before rectal administration.

4. Conclusion

Based on the results obtained in vivo, absolute bioavailability of quinine hydrochloride in rectal aqueous solution can be increased from 62.7 to 86.3% in rabbits using a MA gel. The lower bioavailability of quinine from the TS gel could be likely due to both its weak mucoadhesive properties and its high viscosity. Thus, TS and MA gels appeared to have similar tolerability on the rectal mucosa of the rabbit. However, the MA gel appeared to be more effective in terms of bioavailability than TS gel. Therefore, the MA gel, which will be much easier to produce on a large scale and to store in tropical countries than the TS gel, seems more appropriate to be used in hot countries and a better choice to reach the goal of this work. Nevertheless, bioavailability assessment on human subjects experiencing malaria attack should be carried out on the both rectal gels.

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