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Research paper

In vitro and *in vivo* characteristics of a thermogelling and bioadhesive delivery system intended for rectal administration of quinine in children

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Abstract

The aim of this work was to improve the rectal bioavailability of quinine hydrochloride by designing thermosensitive and mucoadhesive gels intended for rectal delivery. The rheological and mucoadhesive properties of poloxamer 407 solutions have been modulated by addition of hydroxypropylmethycellulose (HPMC) and propanediol-1,2. *In vitro* release and rectal absorption of quinine have been highlighted by a dialysis dissolution testing method and by the determination of bioavailability of the different formulations in rabbits. Increasing the proportions of HPMC and poloxamer in the formulations resulted in a prolonged release of quinine. Indeed, compared to the DT 50% of a rectal solution and a simple HPMC gel (27 and 65 min, respectively) the DT 50% of thermosensitive ternary systems was increased and ranged between 80 and 138 min, depending on the system composition. The release rate depended strongly on the elasticity of the gels after thermogelation. The absolute rectal bioavailability of quinine determined in rabbits was significantly improved with these thermosensitive and adhesive systems. It increased from 62% for the rectal solution to 98% for a ternary system 16/0.5/30 (poloxamer (16%)/HPMC (0.5%)/propanediol-1,2 (30%)). As a result of combined bioadhesion and prolonged release of quinine *in vivo*, higher average values of MRT and t_{max} (9.1 ± 0.2 h and 30 min, respectively) were obtained compared to the rectal solution (6.9 ± 0.9 h and 15 min, respectively). Moreover, these formulations presented a very good rectal tolerance.

Modulation by HPMC of the viscoelastic and mucoadhesive properties of poloxamer 407 thermogelling solutions allowed a prolonged release of quinine hydrochloride and an improvement of bioavailability in rabbit.

Keywords: Rectal delivery; Bioavailability; Bioadhesion; Quinine; Poloxamer; HPMC

1. Introduction

The rectal route offers many advantages such as a very weak enzymatic degradation of drugs because of the very limited presence of proteolytic enzymes and other enzymes in the rectum, a partial avoidance of the first hepatic passage, transport by the lymphatic system of a large quantity of drug, a constant and static environment, a perfect availability for the patients who have problems of vomiting and nauseas and finally it is a way of avoiding. Taking account of all these advantages and because of a very important venous irrigation of the rectal mucous membrane, the rectal route could be used as an emergency route and as an alternative to the parenteral route. Indeed, the use of the rectal route avoids the side effects caused by the use of parenteral injections such as microbiological contaminations, which are quite frequently occurring in developing countries resulting in poliomyelitis,

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abscesses, hepatitis and gas gangrenes [1–4]. Therefore, in some cases, the rectal route would be a route of choice provided that convenient and efficient dosage forms can be formulated. Ideally, rectal dosage forms should remain long enough in the rectum without being ejected for ensuring maximal absorption of the drug [5]. For this purpose, a possible strategy consists in imparting bioadhesive properties to the formulation, either to improve a local effect or to enhance drug absorption [6–11]. Such a strategy has already been successfully attempted for improving rectal absorption of drugs [12,13]. However, it should be kept in mind that in addition to the immobilization of the dosage form, drug release has to be modulated for occurring and being completed during the immobilization period, making the formulation of such systems quite complicated.

It was the aim of this work to develop a bioadhesive and thermogelling system intended for improving the rectal administration of quinine. Such a dosage form would be of therapeutic interest to avoid the difficulties encountered in the emergency treatment of infantile serious paludism. Presently, for such a therapeutic indication, quinine is administered in the form of parenteral solutions, with many limitations, as discussed above. For this reason, in practice these solutions are also administered by the rectal route by physicians. These last years, some therapeutic protocols for the treatment in acute urgency of paludism in the children reported that injectable Quinimax® appeared effective by the rectal route [14,15]. However, the bioavailability was obstructed by the loss of a part of the liquid solution during or following the rectal introduction and some inflammatory effects resulting in bleeding diarrhoeas were also reported [14]. In a previous work, we have reported the formulation of a thermogelling solution based on the use of poloxamer 407 as a thermogelling agent [16]. This polymer was highly chemically compatible with quinine hydrochloride and very stable gels could be obtained. Indeed, in the case of the rectal route, it would be benefitting to have systems that could be handled in the liquid state before their delivery. This is likely to facilitate their use in children and to increase the surface of contact with the mucous membrane. But after thermogelation, these preparations revealed to be not sufficiently adhesive and not rigid enough to remain for a long time in the rectum. For these reasons, the aim of the present work consisted in the design of a bioadhesive and thermogelling system for controlling the release of quinine during a sufficiently long time in the rectum. For this purpose ternary systems containing poloxamer 407, a highly viscous grade of hydroxypropylmethycellulose (HPMC 4KM) and propanediol 1, 2 in various proportions were formulated and characterized. Their *in vitro* release characteristics, as well as their pharmacokinetics, in rabbits were determined. These data were discussed in view of the textural characteristics of the preparations, including their viscoelastic characteristics and mucoadhesive properties.

2. Materials and methods

2.1. Materials

Hydroxypropylmethycellulose (HPMC) (Methocel K4M series MM87091702 K) was a gift from Colorcon. Poloxamer 407 (Lutrol F 127 series No. 09603401 and batch No. 49-0023) was gracefully offered by BASF. Propanediol-1, 2 (Prolabo, Paris, Lot No. 88 208), quinine hydrochloride (Cooper, Melun, France) and lactic acid (Sigma, St. Louis, MO) were used as received. All other reagents were of analytical grade.

2.2. Preparations

The intravenous solution used for pharmacokinetics was prepared just before administration. Briefly, 4.8 g of quinine hydrochloride (equivalent to 4 g of quinine base) was dissolved in sterile distilled water (100 g). After quinine hydrochloride was completely dissolved, the intravenous solution was filtered through a membrane filter (pore size 0.22 μm). The pH value of this solution was 6.3. The rectal solution was prepared by addition of 4.8 g of quinine hydrochloride at room temperature to 7 g of a 0.5% lactic acid aqueous solution and 59.2 g of deionised water under magnetic stirring until a clear solution was obtained. Propanediol-1,2 (30 g) was finally added to this solution.

The different rectal gels were prepared by using a mixer equipped with a turbin adapted to the mixing of viscous preparations (Rayneri-turbotest, Rayneri, France) either at room temperature or in an ice bath. HPMC powder was gradually added under agitation (1000 rpm) to a liquid phase, which consisted in a mixture of an aqueous solution of quinine hydrochloride, propanediol-1, 2 30% (w/w) and lactic acid 0.5% (w/w). Poloxamer 407 was then added gradually under continuous agitation (1000 rpm) until total dissolution. Depending on the final viscosity of the preparation and the ratio of HPMC and poloxamer, poloxamer could be dissolved first and HPMC at last. Finally, the pH of the preparations was adjusted to 5.3 with lactic acid to avoid any precipitation of quinine. The final concentration (expressed in quinine base) was 40 mg/g of preparation. The mixture was then placed at $T = +4$ °C during 48 h to eliminate foam and air bubbles and the macroscopic homogeneity of each sample was appreciated visually at the end of the storage period. A viscous solution was obtained, which was finally homogenized by gentle agitation during 15 min before characterization experiments. The different rectal gels were denominated by three numbers indicating the w/w percentage of poloxamer 407, HPMC and propanediol-1, 2, respectively.

2.3. Rheological studies

Rheological measurements were carried out on a CSL 100 controlled stress rheometer (Carri-Med, Rhéo, Champlan, France). The geometry was a stainless steal cone/

plate (diameter 4 cm, angle 3°58′ and gap 110 μm) which was equipped with a solvent trap to avoid evaporation during measurement. Oscillatory experiments were carried out. A sinusoidal shear was applied to the samples. From the phase angle between the shear stress and the deformation, one could define the elastic (or storage) modulus G′ and the viscous (or loss) modulus G″. Thanks to Peltier diodes which were placed in the lower plate, temperature sweeps from 0 to 80 °C could be performed with a precision of 0.1 °C. Thus, it was possible to determine the sol–gel transition temperature T_{gel} by measuring the temperature for which G′ underwent a critical variation at a shear frequency of 1 Hz. The gel texture was also characterized at $T = 37$ °C (beyond the gel point), by recording the G′ and G″ variations as a function of shear frequency. These experiments were carried out under a stress value of 60 Pa which belonged to the viscoelastic linear domain where the sample did not undergo irreversible structural modifications. All rheological results are the mean of $n = 5$ experiments.

2.4. Mucoadhesion studies

The experimental procedure used for determining the mucoadhesion of the different preparations was previously reported [17]. Briefly, a hydrogel layer (10 mm in height, contact surface: 3.8 cm^2) was placed in contact with a fragment of freshly excised rabbit rectal mucosa under thermostated conditions, thus forming an adhesive joint between the two surfaces. After a preset contact time (10 min) under an initial contact strength (0.5 N), the two surfaces were separated at a constant displacement rate of 1 mm/s using a texture analyser (TAXT2, Stable Micro System, Rhéo, Champlan, France). The strength was recorded as a function of the displacement, which allowed to determine the maximal detachment force (F_{max}) and the work of adhesion (W) which was calculated from the area under the strength – displacement curve. F_{max} and W values were the mean of $n = 6$ experiments performed at $t = 37$ °C.

2.5. In vitro drug release kinetics

The experimental technique used for the *in vitro* release study of quinine was derived from a previously published method [16]. Briefly, *in vitro* release of quinine from the rectal formulations was monitored using the USP basket apparatus at a rotating speed of 100 rpm in 1000 ml of deionised water at $T = 37.0 \pm 0.5$ °C. An automatic dissolution-rate system (Sotax AT 7, Switzerland) suitable for continuous spectrophotometric analysis was used. 2 g of each formulation was introduced in a Spectra/pore® dialysis tubing (cut off: 6000 Da) after being tightly tied at one end. Then, the other end was sealed to obtain an approximately spherical bag which was immediately put into a basket. For each formulation six bags were prepared and tested. Absorbance was measured at 235 nm over 6 h. The rectal solution of quinine hydrochloride was used as a 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 6 h according to Khan's method [18,19].

2.6. Pharmacokinetic study

2.6.1. Animals

Experiments were carried out on male albinos rabbits (Elevage du Vivalium, ENS, Côte d'Ivoire) 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 six groups (six animals per group). One group received a quinine solution intravenously. The five other groups were given five distinct quinine rectal formulations (one formulation each group). All experiments on animals adhered to the Communities Commission Directive 87/302/EEC (EEC, 1987) and were performed in conformance with the French Ministry of Agriculture Permission No. 03640.

2.6.2. Administration and blood samples collection

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 4 cm silicon tube (6 mm external diameter, 4 mm internal diameter). 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 the quinine plasmatic concentration

Following administration of the different quinine formulations, 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 4 °C until further processing. Extraction of quinine was carried out according to the method of Nielsen et al. [20]. Briefly, 250 μl of plasma was mixed with 25 μl of 1 M NaOH. Quinine was then extracted with 3 ml of methylene chloride. The mixture was centrifuged at 1500 rpm. After elimination of the aqueous phase, 2 ml of methylene chloride was transferred into a glass tube and evaporated to dryness at 45 °C. The residue was then dissolved in 1 ml of mobile phase. A 50 μl aliquot was injected onto the column. This last operation was carried out in triplicate. The HPLC equipment consisted of a Waters® 501 pump and a Waters® 712 WISP Autosampler (Waters, Saint-Quentin en Yvelines, France), a Waters® 474 fluorimetric detector and a chromatography work-station Baseline 810 (Waters). Separation was achieved at room temperature on a 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 pH 2.5 sodium perchlorate 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 an emission wavelength of 430 nm.

2.6.4. Pharmacokinetics

C_{max} and t_{max} were determined from the plasmatic profiles following rectal delivery of the different formulations. 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 infinite time according to Eq. (1):

$$AUC_{10-\infty} = \frac{C_{10}}{k_e} \quad (1)$$

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 follows:

$$Cl_T = \frac{dose \cdot F}{AUC_{0-\infty}} \quad (2)$$

and

$$V_d = \frac{Cl_T}{k_e} \quad (3)$$

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 from Eq. (4):

$$MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}}, \quad (4)$$

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 calculating the difference between MRT of each rectal formulation and MRT after intravenous administration.

The constant of absorption (k_a) was defined as follows:

$$k_a = \frac{1}{MAT} \quad (5)$$

2.7. Tolerability study

This investigation, which was conducted according to the method of William et al. [21], was carried out on two groups of rabbits. The first group consisted of all rabbits used in the pharmacokinetic study and which received a single dose of 12 mg/kg by rectal route. The second group consisted of rabbits, four rabbits per formulation, having received two doses of 12 mg/kg by rectal route in a 6 h interval. All rabbits were previously prepared as described in the pharmacokinetic study. The rabbits of the first group were sacrificed after 10 h of incubation, therefore at the end of pharmacokinetic experiments, while those of second group were sacrificed 4 h after the second administration. The pentobarbital with 60 mg/kg was used to sacrify the rabbits.

The abdomen was open all along the median-line and 5 cm of distal colon was located and collected. The tissue was open along the mesenteric border and rinsed in warm Krebs solution to eliminate the faeces. The mucosal surface was spread in the Krebs solution. Segments of 3 cm length and 3 mm width in the longitudinal axe were extracted. The mucosal surface was then examined for the detection of superficial macroscopic lesions using a low-power stereo microscope. These observations were compared with the ones obtained on the tissues collected under identical conditions on control animals.

2.8. Statistics

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

3. Results and discussion

3.1. Rheological and mucoadhesive properties

Rectal gels with various amounts of Poloxamer 407, HPMC and propanediol-1, 2 were formulated in order to modulate their rheological and mucoadhesive properties.

Our aim was to obtain formulations which are liquid at room temperature, which exhibit a thermogelation between room temperature and body temperature and which have sufficient adhesive properties to adhere to the rectal mucous membrane. In most tropical countries where paludism prevails, room temperature generally ranges between 25 and 33 °C.

These rectal gels were characterized by their macroscopic aspect, their gelation temperature T_{gel} and their rheological and adhesion parameters determined at $T = 37$ °C. The results of this study are gathered in Table 1. Preliminary experiments have allowed optimization of amounts of poloxamer, propylene glycol and HPMC to be used (data not shown). In the absence of Poloxamer 407, sample 0/0.5/30 did not present any gelation and displayed no adhesion on the rectal mucosa of rabbit. Two poloxamer 407 concentrations were studied, which were selected as follows. The systems with concentrations below 16% did not gel and the systems with concentrations above 17% were difficult to prepare because poloxamer 407 did not dissolve completely. From the results of Table 1, it appears that at a given Poloxamer 407 concentration, a small increase in the amount of HPMC resulted in decreased T_{gel} values and in increased elasticity at 37 °C (higher values of

Table 1
Macroscopic aspect, gelation temperature T_{gel}, elastic modulus G′ and viscous modulus G″ (at shear frequencies of $2 \cdot 10^{-2}$ and 1 Hz), work of adhesion W, and maximal detachment force F_{max} for different quinine-loaded rectal gels (40 mg/g of preparation)

Rectal gel composition	Aspect	T_{gel} (°C)	G′ (10^3 Pa) at $2 \cdot 10^{-2}$ Hz	G″ (10^3 Pa) at $2 \cdot 10^{-2}$ Hz	G′ (10^3 Pa) at 1 Hz	G″ (10^3 Pa) at 1 Hz	W (mJ/cm^2)	F_{max} (N/cm^2)
0/0.5/30	Homogeneous and limpid	N.G.	$(0.5 \pm 0.1) \cdot 10^{-5}$	$(6 \pm 3) \cdot 10^{-5}$	$(0.8 \pm 0.1) \cdot 10^{-3}$	$(3.5 \pm 0.1) \cdot 10^{-3}$	N.A.	N.A.
16/0/0		34 ± 2	0.4 ± 0.1	1.1 ± 0.3	6.2 ± 0.7	2.0 ± 0.3	N.A.	N.A.
16/0/20		29 ± 2	0.1 ± 0.1	0.6 ± 0.5	6.5 ± 0.7	2.6 ± 0.2	N.A.	N.A.
16/0/30		N.G	$(1.2 \pm 0.1) \cdot 10^{-7}$	$(7 \pm 2) \cdot 10^{-7}$	$(5 \pm 3) \cdot 10^{-5}$	$(9 \pm 4) \cdot 10^{-5}$	N.A.	N.A.
16/0.5/30	Homogeneous and creamy	33 ± 2	1.5 ± 0.2	1.8 ± 0.1	6 ± 1	1.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.2
16/1/30		30 ± 1	1.9 ± 0.3	2.2 ± 0.2	8 ± 2	1.7 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
16/1.5/30		24 ± 2	5 ± 1	1.9 ± 0.3	11 ± 1	1.5 ± 0.3	0.4 ± 0.2	0.3 ± 0.1
17/0/0	Homogeneous and limpid	32 ± 2	3.2 ± 0.4	2.7 ± 0.2	7.5 ± 0.5	1.6 ± 0.1	N.A.	N.A.
17/0/20		22 ± 2	1.5 ± 0.1	2.3 ± 0.3	10 ± 1	2.2 ± 0.2	N.A.	N.A.
17/0/30		N.G	$(2 \pm 0.1) \cdot 10^{-7}$	$(10 \pm 2) \cdot 10^{-7}$	$(10 \pm 3) \cdot 10^{-5}$	$(15 \pm 3) \cdot 10^{-5}$	N.A.	N.A.
17/0.5/30	Homogeneous and creamy	26 ± 4	4 ± 1	1.9 ± 0.2	9 ± 2	0.9± 0.1	0.3 ± 0.1	0.3 ± 0.1
17/1/30		18 ± 1	7 ± 2	2.1 ± 0.1	13 ± 1	1.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
17/1.5/30		11 ± 2	11 ± 3	3.1 ± 0.4	16 ± 1	1.7 ± 0.1	0.4 ± 0.1	0.3 ± 0.2

$n = 5$. (Excepted macroscopic aspect and gelation temperature, all parameters have been determined at 37 °C). N.G., no gelation; N.A., no adhesion.

G′). Whereas the 16/0/30 and the 17/0/30 systems displayed no adhesion, the ternary systems exhibited good and approximately equivalent adhesive properties in the HPMC concentration range (0.5–1.5%). Besides, these various rectal gels exhibited different viscoelastic behaviours (see Table 1). Indeed, rectal gel 0/0.5/30 containing no poloxamer had a viscous character (G″ > G′) at both frequencies ($2 \cdot 10^{-2}$ and 1 Hz) and behaved as a liquid. As a consequence, this system was not cohesive enough to present any measurable adhesion properties on the rectal mucosa of rabbits. For rectal gels 16/0.5/30 and 16/1/30 a transition was observed between a predominantly viscous behaviour (G″ > G′) at $2 \cdot 10^{-2}$ Hz and a predominantly elastic behaviour at 1 Hz (G″ < G′). With the four other mixtures (16/1.5/30, 17/0.5/30, 17/1/30 and 17/1.5/30) the elastic properties were very pronounced at both frequencies. Obviously, the rheological and the mucoadhesive properties are strongly correlated and gel elasticity is requested for promoting efficient bioadhesion. These structural relationships were analyzed in detail in a previous work [17].

Among the ternary systems exhibiting mucoadhesive properties, only the systems 16/0.5/30, 16/1/30 and 17/0.5/30 were transformed into gel with T_{gel} ranging between 30 and 37 °C. Thus, these systems were interesting as they could easily be administrated in the liquid state at room temperature and would gel at body temperature. However, the other systems (16/1.5/30, 17/1/30 and 17/1.5/30) gelled at temperatures lower or equal to 24 °C and would be gelified at room temperature under tropical climate.

3.2. In vitro drug release kinetic

Release profiles of quinine from the various formulations are shown in Fig. 1 and the main release kinetic characteristics are gathered in Table 2. As expected, the experimental dialysis setup resulted in a pseudo "release profile" in the dissolution medium (DT 50%), due to the slowdown effect of the dialysis membrane (cut-off = 6000 Da, compared to the molecular weight (MW) of quinine 324 Da). Compared to the rectal solution (DT 50% = 27 min), quinine release was slowed down by addition of HPMC (DT 50% = 65 min for rectal gel 0/0.5/30) and even more by the simultaneous addition of Poloxamer 407 and HPMC. Ryu et al. [13], by associating mucoadhesive substance (sodium alginate, polycarbophil, carbopol, poly(vinyl pyrrolidone) and hydroxypropylcellulose) to a mixture of poloxamers 407 and 188, also showed that these substances slowed down the *in vitro* release of propanolol.

Among the ternary systems, the release rate of quinine was the highest (DT 50% = 80 min) for the rectal gel 16/0.5/30 which had the lowest Poloxamer 407 and HPMC concentrations and was the lowest for rectal gel 17/1.5/30 which had the highest polymer concentrations. The same trends were observed with the cumulative amounts of released drug (CARD) over the first 3 and 6 h. These

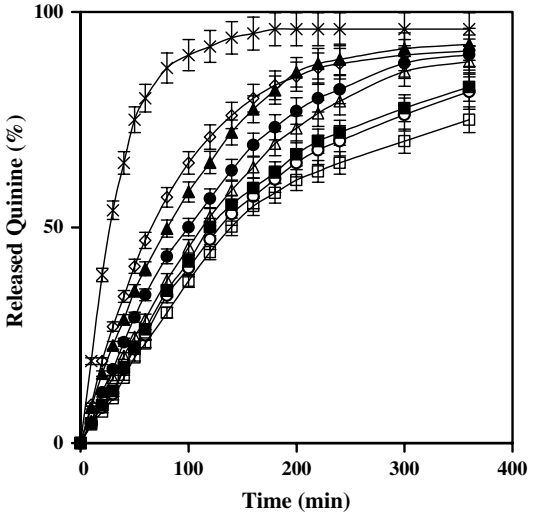

Fig. 1. Release profiles of quinine in deionised water (pH 6.9) and for the rectal gels 0/0.5/30 (◇), 16/0.5/30 (▲), 16/1/30 (●), 16/1.5/30 (■), 17/0.5/30 (△), 17/1/30 (○) and 17/1.5/30 (□), for the rectal solution (×). $n = 6$, mean ± SD.

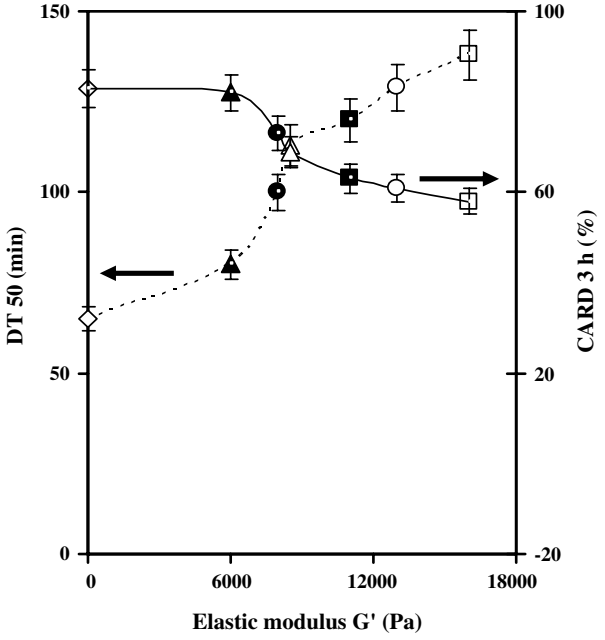

Fig. 2. Variations of DT 50% and CARD 6 h versus the elastic modulus G′ at 1 Hz for the rectal gels 0/0.5/30 (◇), 16/0.5/30 (▲), 16/1/30 (●), 16/1.5/30 (■), 17/0.5/30 (△), 17/1/30 (○) and 17/1.5/30 (○). $n = 6$, mean ± SD.

results were consistent with the decrease of the quinine diffusivity within the system when the total polymer concentration increased. Release characteristics were also strongly correlated to the elastic characteristics of the gels. Fig. 2 shows unambiguously that quinine release was slowed down when the gel elasticity was increased.

3.3. Pharmacokinetic study

Three ternary systems likely to be of interest on the clinical level and which were liquid at temperatures ranging between 25 and 30 °C, i.e. the systems 16/0.5/30, 16/1/30 and 17/0.5/30, were retained for these pharmacokinetic tests. They were compared to the intravenous quinine solution, to the rectal quinine solution and to the simple HPMC (0/0.5/30) gel.

Fig. 3 displays the profiles of the mean plasma concentration of quinine versus time obtained either after rectal administration of the different rectal gels and solution, or after intravenous administration of the quinine solution. The main pharmacokinetic parameters are summarized in Table 3.

As expected, C_{max} and t_{max} values were affected by the controlled released of quinine from the systems. C_{max} for the three ternary systems 16/0.5/30, 16/1/30 and 17/0.5/30 were equal to 853 ± 108, 810 ± 117 and 805 ± 180 ng/ml, respectively, but were not significantly different ($P < 0.0001$). They were lower than C_{max} for the rectal gel 0/0.5/30 (1310 ± 180 ng/ml). The quinine prolonged release due the simultaneous presence of HPMC and poloxamer could also be noticed when analyzing the t_{max} values. Indeed the ternary systems had a t_{max} of 30 min, which was twice higher than the t_{max} of the 0/0.5/30 gel and of the rectal solution (15 min). The mean absorption time (MAT) increased from 4.8 to 7.7 h for the rectal solution and the 17/0.5/30 gel, respectively. Simultaneously, the absorption constant k_a was reduced in the case of the rectal gels, compared to the rectal solution. Similarly, MRT found for all

Table 2
In vitro release characteristics of quinine in deionised water at pH 6.9 from the different rectal formulations ($n = 6$)

Dissolution characteristics	Rectal solution	Rectal gel composition (poloxamer 407%/HPMC%/propanediol-1,2%)						
		0/0.5/30	16/0.5/30	16/1/30	16/1.5/30	17/0.5/30	17/1/30	17/1.5/30
DT 50% (min)	27 ± 1	65 ± 2	80 ± 5	100 ± 3	120 ± 7	113 ± 6	129 ± 3	138 ± 7
DT 80% (min)	60 ± 3	160 ± 2	170 ± 4	220 ± 3	330 ± 1	250 ± 2	340 ± 2	> 360
CARD 3 h (%)	94 ± 2	83 ± 3	82 ± 3	73 ± 1	63 ± 6	69 ± 4	61 ± 5	58 ± 1
CARD 6 h (%)	96 ± 1	91 ± 1	92 ± 9	90 ± 2	83 ± 8	88 ± 4	82 ± 3	75 ± 1
DE 6 h (%)	86 ± 1	71 ± 4	70 ± 5	64 ± 1	55 ± 1	60 ± 4	54 ± 5	50 ± 2

DT 50%, dissolution time of 50% of the initial quinine sample content; DT 80%, dissolution time of 80% of the initial quinine sample content; CARD 3 h, cumulative amount of released quinine over the first 3 h; CARD 6 h, cumulative amount of released quinine over the first 6 h; DE 6 h (%), dissolution efficiency calculated over 6 h.

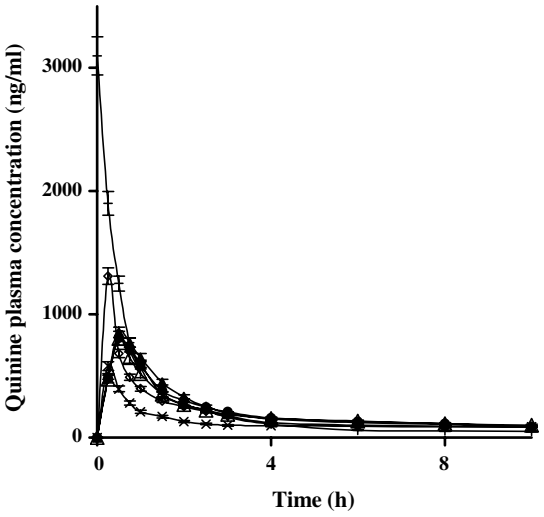

Fig. 3. Variations of the plasma concentration of quinine versus time after intravenous bolus administration or rectal administration. Intravenous solution (+), rectal solution (×), rectal gels 0/0.5/30 (◇), 16/0.5/30 (▲), 16/1/30 (●) and 17/0.5/30 (△). $n = 6$, mean ± S.D.

ternary systems was higher than MRT for the gel 0/0.5/30 (8.8 ± 0.8 h), containing only HPMC and were also significantly higher than the MRT (6.9 ± 0.9 h) obtained after rectal administration of the quinine solution. All these data suggest a sustained-release of quinine from the ternary systems and are in qualitative agreement with the *in vitro* release study results (see Section 3.2).

In comparison with the $AUC_{0-\infty}$ obtained for the rectal solution of quinine (1728 ± 75 ng h ml^{-1}), $AUC_{0-\infty}$ for the ternary systems was significantly higher ($P < 0.0001$). In comparison with the $AUC_{0-\infty}$ of gel 0/0.5/30 containing only HPMC, one observed an increase of about 6% to 8%. Among the four rectal gels, $AUC_{0-\infty}$ for the system 16/0.5/30 was the highest (2700 ± 197 ng h ml^{-1}). However, the $AUC_{0-\infty}$ for the three ternary systems was not significantly different, which could be explained by their relatively similar viscoelastic and adhesive properties.

Thus, the highest bioavailabilities, either absolute bioavailability (F) or relative bioavailability (F'), were obtained for the ternary systems with F close to 100% and F' in the range of 150%. On the other hand, the bioavailability of the HPMC gel (0/0.5/30) containing no poloxamer was 91%, despite the fact that the absorption constants (k_a) were similar for the different rectal gels, which suggests that other characteristics of the ternary gels, such as mucoadhesive properties, had positive effects on quinine bioavailability.

The controlled release of quinine from the gels resulted in an increase of the apparent elimination half-life, which was significantly increased ($P < 0.0005$) compared to the rectal solution (3.6 ± 0.6 h) or the rectal gel containing no poloxamer (0/0.5/30). No significant difference was found between the mean $t_{1/2}$ values corresponding to the different gels containing poloxamer. V_d for the rectal gels was significantly higher than V_d ($P < 0.0002$) found for the intravenous administration (18 ± 3 l kg^{-1}) and V_d ($P < 0.002$) for the rectal solution (22 ± 2 l kg^{-1}). Similar increases in the apparent $t_{1/2}$ have been shown previously in some situations when using prolonged release gels [16,22]. In this latter example [22], the second elimination half-life of vancomycin from poloxamer 407 gels intended for treating prosthesis infections, was considerably increased, when compared to the solution.

No significant difference was found between the total plasma clearance Cl_T after intravenous administration of quinine solution (4.4 ± 0.4 l h^{-1} kg^{-1}) and those obtained after rectal administration of rectal gels and solution. It can be deduced that rectal gels did not affect the rate of the elimination process.

Table 3
Bioavailability and pharmacokinetic parameters ($n = 6$, mean values ± S.D.) following the intravenous or rectal administration of quinine formulations (12 mg of quinine base/kg) to 6 groups of rabbits (one formulation per group)

Pharmacokinetic characteristics	Intravenous solution[a]	Rectal preparations				
		Rectal solution[b]	Rectal gel composition (poloxamer 407%/HPMC%/propanediol-1,2%)			
			0/0.5/30	16/0.5/30	16/1/30	17/0.5/30
C_{max} (ng ml^{-1})	–	580 ± 97	1310 ± 180	853 ± 108	810 ± 117	805 ± 142
t_{max} (h)	–	0.3	0.3	0.5	0.5	0.5
AUC_{0-10} (ng h ml^{-1})	2610 ± 92	1260 ± 95	1929 ± 101	2213 ± 171	2153 ± 144	2068 ± 90
$AUC_{0-\infty}$ (ng h ml^{-1})	2750 ± 112	1728 ± 75	2509 ± 185	2700 ± 197	2670 ± 186	2665 ± 131
F (%)[a]	100	62.8	91.3	98.2	97.1	96.9
F' (%)[b]	–	100	145.2	156.2	154.5	154.2
k_e (h^{-1})	0.34 ± 0.03	0.19 ± 0.02	0.12 ± 0.02	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.02
$t_{1/2}$ (h)	2.2 ± 0.2	3.6 ± 0.6	5.9 ± 0.6	5.9 ± 0.7	5.9 ± 0.7	6.0 ± 0.5
MRT (h)	2.2 ± 0.3	6.9 ± 0.9	8.8 ± 0.8	9.1 ± 0.2	9.4 ± 0.3	9.9 ± 0.2
MAT (h)	–	4.8	6.5	6.9	7.2	7.7
k_a (h^{-1})	–	0.21	0.15	0.14	0.14	0.13
Cl_T(l h^{-1} kg^{-1})	4.4 ± 0.4	4.3 ± 0.5	4.4 ± 0.6	4.4 ± 0.6	4.5± 0.4	4.6 ± 0.6
V_d (l kg^{-1})	18 ± 3	22 ± 2	37 ± 4	38 ± 5	39 ± 3	40 ± 3

C_{max}, maximum plasma concentration; t_{max}, time to reach C_{max}; F, absolute 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.

[a] Intravenous solution was used as a reference.

[b] Rectal solution was used as a reference.

As a conclusion, pharmacokinetic data strongly suggest that quinine administration could be considerably increased by the mean of simple thermogelling and bioadhesive systems, based on well known excipients. Choi et al. [23] showed an improvement of the rectal availability of acetaminophen in a gel associating two poloxamer (407 and 188) with a bioadhesive polymer (polycarbophil). Here, we obtained a very significant improvement of the rectal bioavailability by using only poloxamer 407 associated to HPMC and in the presence of propanediol-1,2, although the bioadhesive properties of HPMC 4 KM are definitely lower than those of polycarbophil [24].

In the present case, structural modifications of the gels, resulting of an increase in the elasticity of the gels, would be responsible not only for the prolonged release of quinine, but also for improved textural characteristics, including pronounced mucoadhesive characteristics.

Indeed, in rectal administration, the bioavailability of the drugs, especially for those which have an important first pass metabolism as it is the case of quinine, depends on the site of absorption in the rectum and is maximum when the dosage form is close to the anus [25–28]. So the drugs should be administered and located as close as possible to the anus to obtain a maximal effect of first-pass avoidance. This observation is usually explained in terms of venous blood drainage within the rectum. Another advantage of keeping the dosage form in the lower third of the rectum is the possibility of avoiding interferences from faecal matter. In the case of the rectal solution, it is likely that this form can easily migrate to the upper parts of the rectum. On the contrary, the formulated gels are likely to be immobilized by a bioadhesion phenomenon directly at the site of administration, immediately after dosing, due to instant gelation at body temperature. Although, it should be confirmed by imaging techniques, it was indeed likely that the thermogelling gels were immobilized in the first third of the rectum, where first-pass effect is minimal for quinine. Combined to adequate controlled-release properties, excellent bioavailability is probably the result of a favorable combination of these factors *in vivo*.

3.4. Tolerability study

Whatever the gel formulations, all the rabbits received either a single dose (12 mg/kg), or twice the dose (2×12 mg/kg) separated by 6 h. They did not present macroscopic lesions on the rectal mucous membrane as evidenced by visual examination and low magnification binocular examination. Despite these encouraging results, this acute toxicity test should be followed by chronic toxicity tests corresponding to repeated rectal administration of these preparations. Such tests, which are complicated by the difficulty of maintaining rabbits fasted during several days, were out of the scope of this study.

4. Conclusion

Modulation of the viscoelastic and adhesive properties of poloxamer 407 solutions by HPMC in the presence of propanediol-1,2 allowed a prolonged *in vitro* and *in vivo* release of quinine hydrochloride and also an improvement of the rectal bioavailability of this drug in rabbit. Indeed, an absolute bioavailability of quinine close to 100% compared to 62% for a solution was obtained after rectal administration. The viscoelastic characteristics of the gels after thermogelation and simultaneously their mucoadhesiveness were probably responsible for this effect. Additionally, the gelation temperatures of these formulations could be easily modulated to adjust the gelation just below the body temperature. This allows to obtain liquid systems at room temperature which can be conveniently handled and administered and that develop an intimate contact with the rectal mucosa. These unique characteristics make them attractive formulations for improving quinine delivery in diseased children, as they seemed to be more adapted than currently available formulations for the treatment of infantile serious paludism.

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