International Journal of Pharmaceutics, 79 (1992) 29–38

IJP 02604

Poly(diethylmethylidene malonate) nanoparticles as primaquine delivery system to liver

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(Received 22 November 1990)
(Modified version received 4 June 1991)
(Accepted 5 July 1991)

Key words: Diethylmethylidene malonate; Nanoparticles; Primaquine; Drug targeting; Antimalarial activity

Summary

Five analogs of dialkylmethylidene malonate (DAMM) were synthesized with the aim of designing liver primaquine (PQ)-delivery nanoparticles. The diethyl derivative, diethylmethylidene malonate (DEMM), was polymerized in 0.1 M aqueous phosphate buffer and proved to be efficient in adsorbing PQ diphosphate. However, desorption of the entrapped drug was very low. The toxicity in NMRI female mice of the empty and PQ-loaded nanoparticles and of the free PQ diphosphate showed that drug-free nanoparticles were more toxic than PQ-bound nanoparticles whose toxicity was not significantly different from that of free PQ diphosphate. Administration in a single intraperitoneal injection of the free PQ diphosphate, free poly(DEMM) nanoparticles and PQ-loaded poly(DEMM) nanoparticles to *Plasmodium berghei* infected female NMRI mice showed a higher increased life span index (ILS) related with the PQ-loaded nanoparticles.

Introduction

Human malaria is the most important disease in tropical countries. It is caused by four species of *Plasmodium: P. vivax, P. falciparum, P. malariae* and *P. ovale*. The two former species are the most prevalent and account for more than 95% of the malaria in the world. *P. malariae* and *P. ovale* cause mild infections but result in relapsing malaria because of their long-lasting tissue forms which release merozoites into the blood stream over months or years.

It is an infected anopheline mosquito which transmits the parasites to humans: when taking a blood meal, it releases sporozoites into the blood stream. All released sporozoites first invade the parenchymal cells of the liver and subsequently the erythrocytes so that the liver remains the key organ in the course of malaria.

Nowadays, the chemotherapy of malaria must take up several challenges such as drug resistance by various strains of *P. falciparum*, high toxicity

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and low biodisposibility of available drugs, cost and uncertainty of designing new antimalarial drugs.

Among the antimalarial agents, primaquine [PQ; 6-methoxy-8-(4′-amino-1′-methylbutylamino) quinoline] occupies a unique position as the least toxic tissue and blood schizonticide. It is the only drug now in general use against the persistent tissue stages of the relapsing malarias effecting a radical cure.

In an effort to target specifically the liver tissue forms of the parasites and thereby achieve radical cure of relapsing malarias, and to cut short any ulterior development of invading tissue schizonts, the targeting of antimalarial drugs to the liver has been attempted using PQ-peptidyl prodrugs (Trouet et al., 1981; Philip et al., 1988), PQ-protein conjugates (Hofsteenge et al., 1986) and PQ-liposome systems (Pirson et al., 1980). As drug-loaded biodegradable polyalkylcyanoacrylate nanoparticles were found to be entrapped by Kupffer cells (Lenaerts et al., 1984) and due to the simplicity of nanoparticle preparations, their capacity for drug loading (Couvreur et al., 1982), the ready availability of dialkylmethylidene malonate (DAMM) monomers and their ability to polymerise in nanoparticles ranging in size between 100 and 250 nm, we have undertaken PQ loading on colloidal poly(DEMM) nanoparticles as PQ-specific and simpler carriers to the liver.

This paper describes the preparation of PQ-loaded poly(DEMM) nanoparticles and their evaluation in a murine mouse antimalarial model.

Materials and Methods

Synthesis of diethylmethylidene malonate (DEMM) and its analogs

DEMM and its analogs were synthesized as described previously by De Keyser et al. (1988), via retro-Diels-Alder thermolysis of their anthracene adducts. The purity of monomers was determined by GLC analysis on a Hewlett-Packard M 5710A using the following conditions: column, OV17 3%; detector, FID; carrier gas, nitrogen; temperature, 100 °C (2 min) to 270 °C (rate: 10 °C/min). Samples of the monomers are stable for months when kept below 0 °C under nitrogen without any further precautions.

Preparation of free nanoparticles and lyophilization

Nanoparticles were prepared by emulsifying, under mechanical stirring, DEMM monomer (50 μl) in 5 ml of 0.1 M phosphate buffer containing 1% of dextran T 70 (Pharmacia Fine Chemicals, Uppsala, Sweden). The pH of the polymerization medium was kept at 7.4. After 24 h of stirring at ambient temperature (22–25 °C), polymerization was complete and the milky suspension obtained was filtered and dialysed twice against 2 l of 0.1 M phosphate buffer at pH 7.4 for 24 h at 25 °C. Prior to lyophilization, 0.25 g (5%) of glucose (Janssen Chimica, Beerse, Belgium) was added to the suspension which was freeze-dried in a Lyovac GT 2, Leybold Heraus. Samples of the suspension were removed before and after freeze-drying for size determination.

Preparation of PQ-loaded nanoparticles

In situ PQ-loaded nanoparticles In situ drug-loaded nanoparticles were prepared according to the same method as described above, after dissolution of PQ diphosphate salt (Sigma Chemical Co., St. Louis, MO, U.S.A.) at concentrations ranging from 0.5 to 5.0 mg in polymerisation medium (0.1 M phosphate buffer containing 1% of dextran T 70 at pH 7.4). During polymerization at room temperature (22–25 °C), no decomposition of PQ occurred. After 24 h, free PQ concentration was evaluated in the supernatant by withdrawing an aliquot of the yellow milky suspension (5 μl) which was centrifuged at 14000 $\times g$, for 15 min at 15 °C and assayed for PQ content by UV spectrometry. Control of the level of PQ bound to the polymer was carried out after the dissolution of nanoparticles in dimethylformamide. Lyophilization and size determination of drug-loaded nanoparticles were carried out as described above.

PQ-post-loaded DEMM nanoparticles 450 mg of previously lyophilized free nanoparticles, prepared as described above, were successively resuspended with stirring in 5 ml of solutions of PQ diphosphate at concentrations of 0.5–5.0 mg/ml in 0.01 M phosphate buffer at pH ranging from

6.0 to 8.2. The suspensions were kept protected from light and stirred for several days at 20 °C, at ambient temperature (22–25 °C) and at 37 °C. PQ loading was monitored twice per day by withdrawing 5 μl of the suspension, centrifuging it and assaying free PQ in the supernatant. When the adsorption of PQ on the polymer was complete, the yellow suspensions were filtered and lyophilized. Aliquots of the suspensions were removed before and after freeze-drying for size determination.

Nanoparticle characterization and determination of PQ content

The mean size and size distribution of free and PQ-loaded nanoparticles were determined by laser beam light scattering measurement on a Coulter sub-micron particle analyzer (Nanosizer, N4MD, Coulter Electronics Ltd, Luton, U.K.).

PQ content was determined using the supernatant from centrifuged nanoparticles. PQ concentration was calculated as the ratio of the slope of the sample vs the slope of the calibration curve of PQ by UV spectrometry (Perkin Elmer 556, double-beam, double-wavelength, fully automated, fitted with a Hitachi 200-0576 second-derivative device). Analytical conditions were: scanning interval, from $\lambda = 400$ to 200 nm; mode, second derivative; scanning rate, 240 nm per min; scale, abscissa, $2 \times (1\ \text{cm} = 10\ \text{nm})$ and ordinate, 1/10.

Calibration and dilution curves: 2.5 ml of a 1% dextran T 70 solution in 0.1 M phosphate buffer were introduced into the blank and sample cells. Into the sample cell, either 5 μl of a 0.1 mg/ml solution of PQ diphosphate or 5 μl of the supernatant of centrifuged nanoparticle suspension were repeatedly added at increasing concentrations. The absorbances were measured as the height of the PQ absorbance plots and were computed on a Macintosh SE/3o in order to construct, by linear regression, the calibration and sample dilution curves from which the respective slopes (a) and (a') were evaluated. The sample (unknown) PQ concentrations of the supernatant from the nanoparticle suspensions were calculated according to the following equation (Cumps and Derese, 1990).

$$\text{Unknown PC} - \text{cc} = a'/a\ (\mu\text{g/ml})$$

The content of PQ in the polymer was indirectly determined using the relation:

$$\text{PQ}(\%) = 100 - [a'/a\ (\mu\text{g/ml})]$$

where a is the slope of the calibration curve and a' the slope of the sample regression curve.

Drug release from nanoparticles

Drug-loaded previously lyophilized DEMM nanoparticles (450 mg; PQ content, 5 mg) were suspended in either 20 ml of a normal phosphate-buffered saline, PBS (Mishell and Shiigi, 1980), or 20 ml of an 'enzymatic PBS' containing porcine esterase (0.5 ml of 10 mg/ml aqueous suspension, spec. act., 130 U/mg; Boehringer, Germany). Enzymatic PBS was expected to enhance drug release from nanoparticles via enzymatic hydrolysis of the polymer ester functions. The suspensions were incubated at 37 °C at three pH values, namely, 6.0, 7.2 and 7.4, with mild agitation (70 cycles per min). Samples (50 μl) were taken at different time intervals, centrifuged ($14\,000 \times g$, 15 min) and the release of PQ in the supernatant estimated by UV spectrometry. The medium volume was maintained constant by addition of PBS.

Toxicity and antimalarial activity

Acute toxicity Dose-dependent lethal toxicity of free PQ, PQ-DEMM nanoparticles and drug-free DEMM nanoparticles was assessed with female NMRI mice weighing 18–30 g. 10 mice were used per dose of serial drug and particle dilutions. Samples were administered intravenously in a tail vein and intraperitoneally. The data were evaluated by probit transformation and expressed as LD_{50} values according to Lichtfield and Wilcoxon (1949).

Blood schizonticidal activity Free PQ, PQ-loaded nanoparticles and 'empty' nanoparticles were tested for blood schizonticidal activity against *P. berghei* (from the Tropical Medicine Institute, Anvers, Belgium) in female NMRI mice. Batches of 10 mice were injected intraperitoneally with the drugs and NaCl 0.9% (control)

at various time intervals before and after schizont inoculation (−72, −48, −3, +3 h). The doses administered, chosen as the highest non-lethal ones, were as follows: for free PQ, 40 mg/kg; PQ-nanoparticles, 40 mg/kg; empty nanoparticles, 35 mg/kg. Mice were infected intraperitoneally with 1 ml of blood containing about 100 000 parasites per ml. To assess the development of infection, the parasitaemia was checked every 3 days by means of Giemsa-stained thin blood smears. Activity was expressed in terms of the increased life span index (ILS). The ILS was derived from the median survival time (MST) using the relation:

$$\mathrm{ILS} = \left[100 \times \left(\frac{\mathrm{MST\ treated}}{\mathrm{MST\ control}} - 1\right)\right]$$

MST was assessed by recording the length of survival in days of each animal and then calculating the median.

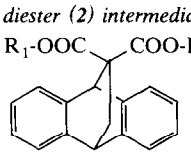
Results

Synthesis of diethylmethylidene malonate (DEMM) and its analogs

The dialkylmethylidene malonate (DAMM) analogs of DEMM were obtained on a multigram scale via a two-step reaction, the first step yielding the methylidene-anthracene adducts listed in Table 1. The subsequent step, Diels-Alder thermolysis, leads to DEMM analogs in good yield

TABLE 1

9,10-Endoethano-9,10-dihydroanthracene-11-dicarboxylic acid diester (2) intermediates in the synthesis of DAMM (1)

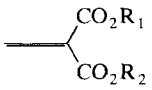


2

Compound	R_1	R_2	Yield (%)
2a	ethyl	ethyl	69.4
2b	allyl	allyl	51.3
2c	ethyl	cyclopentyl	84.1
2d	ethyl	cyclohexyl	51.0
2e	ethyl	cycloheptyl	41.2

TABLE 2

Methylidenemalonic acid diesters (1) obtained after reverse Diels-Alder thermolysis of adducts (2)



1

Compound	R_1	R_2	Yield (%)	Boiling point (°C) [at pressure (Torr)]
1a	ethyl	ethyl	62	63–64 [0.2]
1b	allyl	allyl	46	66–67 [0.3]
1c	ethyl	cyclopentyl	40	70–73 [0.05]
1d	ethyl	cyclohexyl	50	80–82 [0.1]
1e	ethyl	cycloheptyl	30	90–93 [0.05]

and high purity (Table 2). Contamination by maleic anhydride, as measured by GLC, was less than 1% and did not affect the stability of the monomers.

Preparation of free nanoparticles and lyophilization

The mean size of the nanoparticles ranged between 100 and 300 nm (Table 3). The observed distribution was unimodal with a Gaussian profile and consistent with that reported previously for other polymeric nanoparticles (Fig. 1). The size and homogeneity of particles were unaffected by lyophilization.

In situ PQ-loaded nanoparticles

Compared with the post-loading process (see below), in situ PQ binding to nanoparticles occurs rapidly with a maximum being reached after 20 min. As shown in Table 3, PQ concentrations below 2 mg/ml do not affect significantly nanoparticle size or distribution; from 0.5 to 1.5 PQ mg/ml, the mean size of particles ranges between 130 and 100 nm. Similarly, the particle distribution profiles do not differ from that of unloaded nanoparticles (Fig. 1a and b). In contrast, PQ loading at higher drug concentrations resulted in a bimodal size distribution of particles showing a dependence on the PQ concentration (Table 3; Fig. 1c and d): concentrations of 2 mg/ml and higher gave rise to larger nanoparticles and to increasing amounts of microparticles. The appearance these larger particles might result from the PQ-induced colloidal instability of

the nanoparticles, at high concentrations of the drug as observed with polyisohexylcyanoacrylate (PIHCA) nanoparticles (Gaspar et al., 1991).

PQ adsorption onto nanoparticles appears to be concentration dependent as shown in Table 4, however, a PQ concentration of 2 mg/ml in the medium is critical both to the extent of PQ loading which is lower (90%) and to the sizes of the particles which shift to micrometric values. This observation is also in agreement with the findings of Gaspar et al. (1991).

The PQ-loading process was found to be insensitive to incubation temperature (Fig. 2). Similarly, the influence of the pH on the loading process was not significant from 20 to 37 °C at pH values ranging from 5.5 to 8.0 (data not shown). Owing to the chemical instability of PQ solutions at higher pH values, in situ loading of the drug onto the polymer was only achieved at pH 7.4.

PQ-post-loaded DEMM nanoparticles

Preliminary experiments using various PQ concentrations from 0.5 to 5.0 mg/ml showed that drug adsorption onto free poly(DEMM) nanoparticles during the post-loading process was very slow and dependent on the pH, whereas neither the concentration of PQ nor the temperature exerted any influence on the process. Post-loaded nanoparticles presented no bimodal size distribution. Owing to the similarity between the adsorption curves at different values of drug concentration and temperature, only those results relating to a PQ concentration of 1 mg/ml at 37 °C are reported (Fig. 2).

Indeed, at pH 7.4, 81% of the drug initially dissolved in the polymerisation medium is bound to free poly(DEMM) nanoparticles whereas 92% of PQ is retained at pH 8.2. These maximum values for adsorption are reached after several days. The binding of PQ to poly(DEMM)

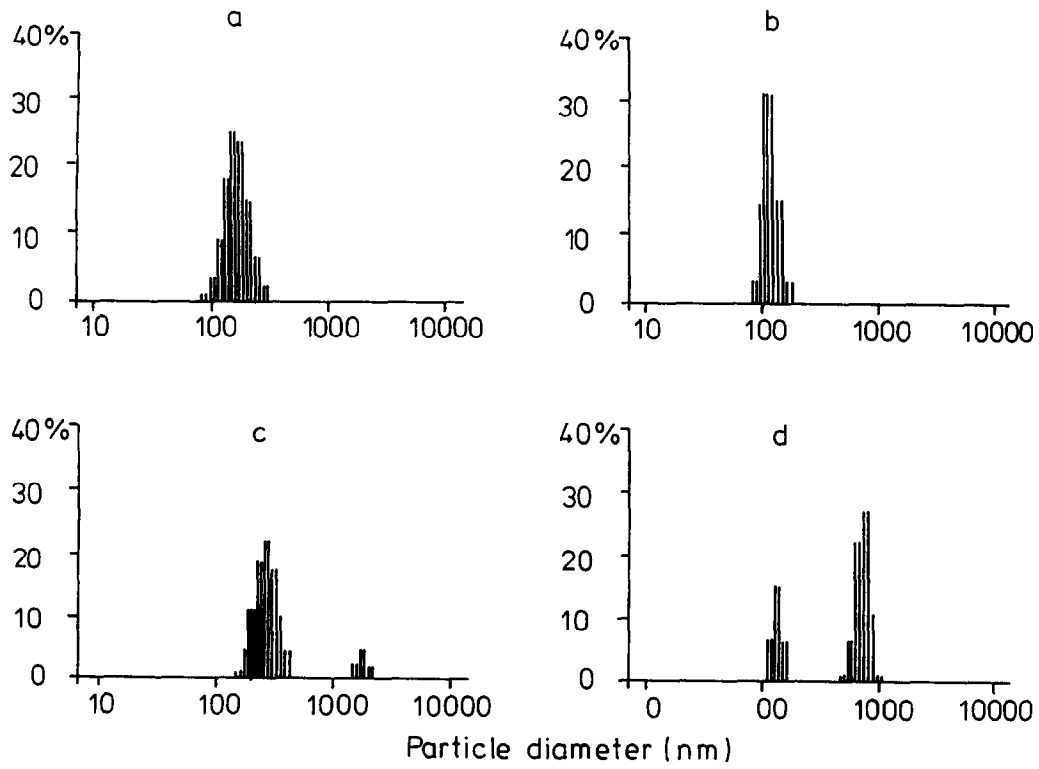


Fig. 1. Size distribution profile of unloaded and in situ PQ-loaded poly(DEMM) nanoparticles prepared at 25 °C and pH 7.4, and at various PQ diphosphate concentrations: (a) 0 mg/ml (unloaded poly(DEMM) nanoparticles); (b) 1 mg/ml; (c) 2 mg/ml; (d) 4 mg/ml.

TABLE 3

Size and distribution of nanoparticles formed with various concentrations of PQ diphosphate

PQ concentration [a] (mg/ml)	Size of in situ PQ-loaded nanoparticles	Distribution [b]	Mean size ± SD (nm)
0.0	100	11	
	178	88	180 ± 59
	316	2	
0.5	100	36	
	178	64	136 ± 28
1.0	100	82	
	178	18	118 ± 32
1.5	100	91	
	178	9	100 ± 32
2.0	178	19	
	316	71	270 ± 73
	562	8	
	1780	10	(1780 ± 210) [c]
4.0	100	15	
	178	14	(133 ± 16) [c]
	562	56	715 ± 110
	1000	14	
5.0	168	40	(168 ± 43) [c]
	1620	60	1620 ± 320

[a] Polymerization medium: 1% dextran T70, 0.1 M phosphate buffer, pH 7.4; temperature, 22–25 °C.
[b] Percentage of PQ-loaded nanoparticles of a given size appearing after polymerization.
[c] Mean size of nanoparticle subpopulations.

TABLE 4

In situ PQ adsorption onto poly(DEMM) nanoparticles with various concentrations of PQ diphosphate

PQ concentration [a]	Adsorption ± SD (%) [b]
0.5	83.3 ± 0.03
1.0	95.2 ± 0.05
2.0	90.0 ± 0.02
4.0	99.2 ± 0.02
5.0	99.6 ± 0.01

[a] Concentration of PQ (mg/ml); medium: 1% dextran T70, 0.1 M phosphate buffer, pH 7.4; 48 h at 22–25 °C.
[b] $n = 5$.

TABLE 5

Percentage of PQ released from poly(MMDE) nanoparticles (I, in situ PQ-loaded nanoparticles; II and III, post-polymerization PQ-loaded nanoparticles)

Incubation time (days)	I [a] ± SD (%)	II [a] ± SD (%)	III [b] ± SD (%)
1	16.2 ± 0.003	17.7 ± 0.004	8.2 ± 0.002
5	16.6 ± 0.005	18.0 ± 0.003	9.1 ± 0.001
10	16.7 ± 0.002	18.2 ± 0.004	9.3 ± 0.003
15	16.6 ± 0.004	18.2 ± 0.004	9.4 ± 0.002
20	16.8 ± 0.003	18.2 ± 0.003	9.4 ± 0.003

[a] Mean of four determinations respectively at pH 6.0 and pH 7.4. Incubation media: PBS and enzymatic PBS at 37 °C.
[b] Mean of four determinations respectively at pH 6.0 and pH 8.2. Incubation media: PBS and enzymatic PBS at 37 °C.

TABLE 6

Blood schizonticidal effect of in situ PQ-loaded nanoparticles, PQ diphosphate and drug-free nanoparticles in NMRI female mice

Injection time (h)	MST ± SD (days) [ILS (%)]			
	PQ-nanoparticles	Free PQ	Free nanoparticles	Control
−72	14.1 ± 6.67	–	–	8.5 ± 3.46
	[64.0]	–	–	–
−24	17.0 ± 6.49	14.1 ± 6.01	8.6 ± 3.47	8.6 ± 3.47
	[98.0]	[63.9]	[0.0]	0.0
−3	12.2 ± 6.9	10.6 ± 6.6	9.3 ± 4.2	9.1 ± 3.70
	[34.1]	[16.5]	[2.2]	0.0
+3	12.25 ± 6.67	12.4 ± 6.69	8.7 ± 3.50	8.6 ± 3.47
	[42.4]	[44.2]	[1.2]	–

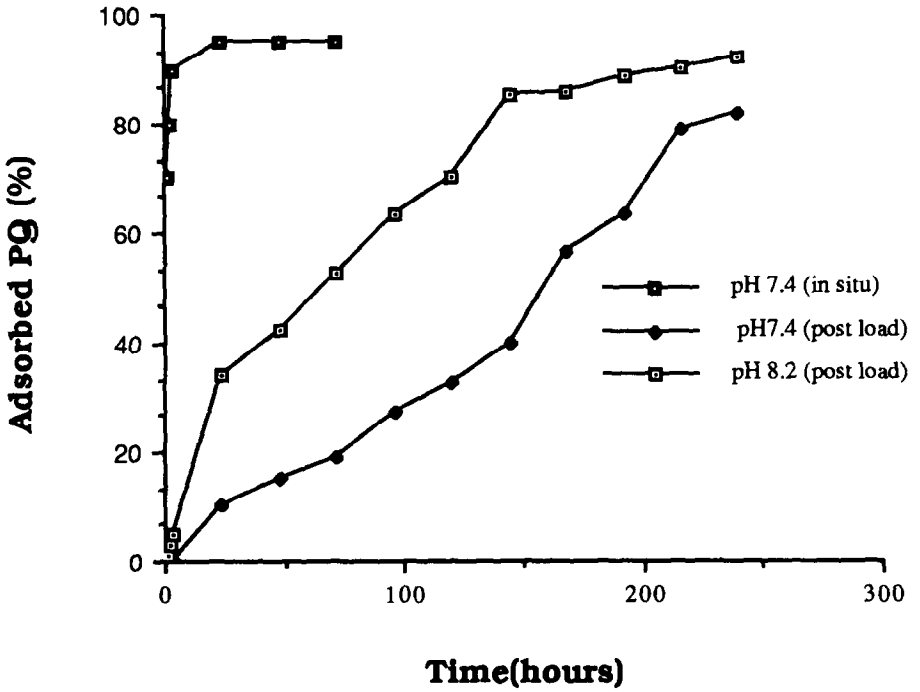


Fig. 2. PQ adsorption onto poly(DEMM) nanoparticles as a function of incubation time and pH. Medium: 0.1 M phosphate buffer, 1% dextran T70, pH 7.4 and 8.2; initial concentration of PQ diphosphate, 1 mg/ml; temperature, 37 ° C.

nanoparticles is faster at pH 8.2: the adsorption half-time (50% PQ binding) is about 60 h at pH 8.2 and 160 h at pH 7.4.

PQ release from nanoparticles

The results shown in Table 5 indicate that the release of PQ in the incubation medium is constant and remains very small in extent. The binding of PQ-poly(DEMM) nanoparticles appears to be quasi-irreversible. The observed concentrations may be attributed to residual drug which had not been entrapped in the nanoparticles during the in situ and post-polymerization adsorption of PQ diphosphate. This may acount for the observation that changing the pH or the addition of esterase had no significant effect on the desorption of PQ.

Acute toxicity

PQ-post-loaded nanoparticles were chemically less stable than those loaded in situ; only the latter were submitted to biological tests. Intravenous acute toxicity of free and PQ-loaded nanoparticles could not be established owing to their considerable lethal effect: 99% of the injected mice died at doses below 10 mg/kg in the case of free poly(DEMM) nanoparticles and 20 mg/kg for drug-loaded (in situ) nanoparticles. The acute toxicity values reported here were assessed by intraperitoneal administration of the drugs. The respective LD_{50} (i.p.) values were determined to be as follows: free PQ, 56.0 mg/kg [53.5–58.6]; PQ-loaded nanoparticles, 58.0 mg/kg [54.2–62.0]; free nanoparticles, 43.0 mg/kg [40.2–44.7].

Blood schizonticidal activity

Table 6 and Fig. 3 display the observed schizonticidal effects of the drugs injected into mice before and after inoculation with parasites. In situ PQ-loaded nanoparticles result in the respective ILS values being higher: 64, 98, 34.1 and 42.4% at −72, −24, −3 and +3 h. Free PQ also improved the ILS of mice at 64.0, 16.5 and 44.2%, respectively, at −24, −3 and +3 h.

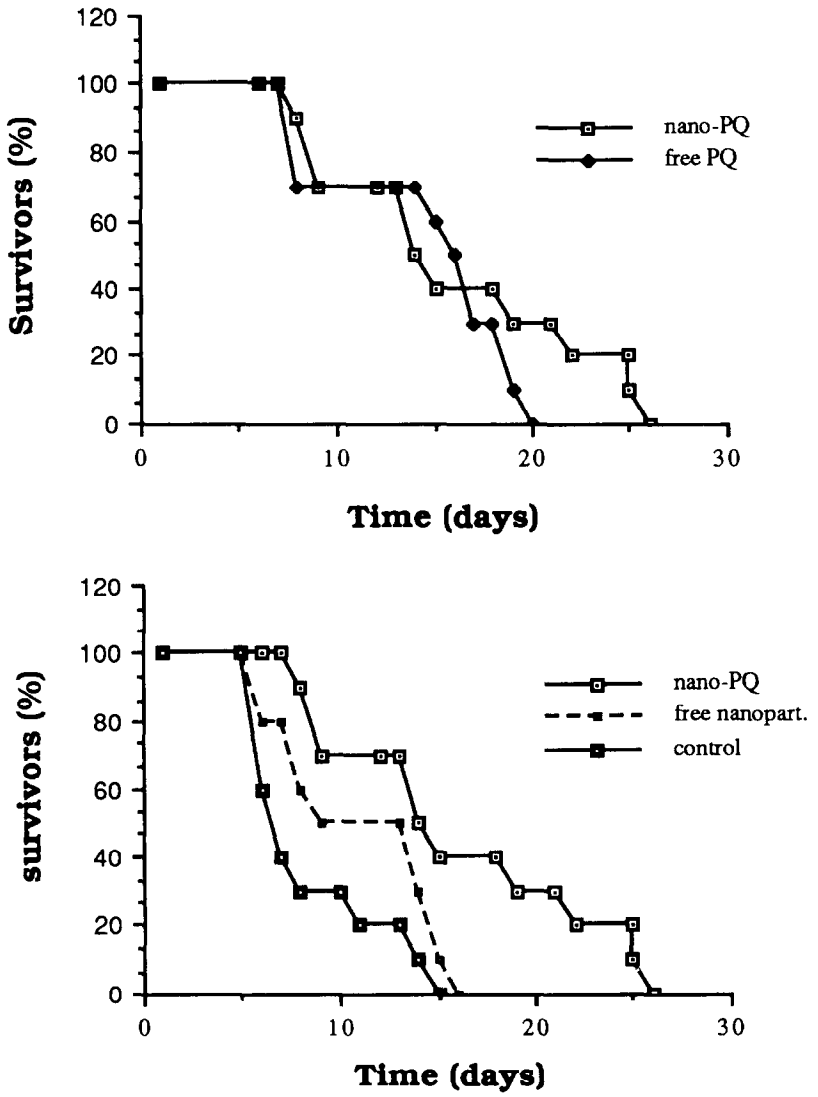


Fig. 3. Blood schizonticidal activity of in situ PQ-loaded nanoparticles (40 mg/kg), and free nanoparticles (35 mg/kg) after schizont inoculation into female NMRI mice.

Discussion

The in situ PQ-loading process on nanoparticles was considered to be optimum at a concentration of 1 mg/ml of PQ diphosphate in the polymerization medium. This value was used throughout our experiments. The sudden appearance of increasing populations of microparticles is consistent with previously reported observations on doxorubicin poly(methylmethacrylate) nanoparticles. This may be attributable to a slight degree of aggregation of nanoparticles under the coating action of the adsorbed drug and to modification of the surface charge of the particles.

Our results demonstrated that, at a concentration of 1 mg/ml of PQ in the polymerization medium, drug loading on poly(DEMM) nanoparticles was effected more readily in the in situ rather than in the post-polymerization approach, which is time-consuming (several days) and detri-

mental to the stability of PQ. The chemical lability, i.e., susceptibility to oxidation, of PQ free base in the light is known and was assessed by Brossi et al. (1987). The high pH used during post-polymerization PQ loading on nanoparticles may account for the fact that these PQ-loaded particles turned brown on storage even when kept under conditions of darkness, dryness and cold. This could be ascribed to the oxidation of PQ as the free base in the presence of traces of various adjuvants: dibasic phosphate, residual monomeric units, etc. In contrast, in situ drug-loaded particles do not become brownish and are very stable on storage over several months.

Comparison of the LD_{50} values of free PQ, PQ-loaded poly(DEMM) and empty poly (DEMM) nanoparticles reveals that the entrapment of PQ in poly(DEMM) nanoparticles does not increase the therapeutic index of PQ diphosphate but does reduce the toxicity of free poly (DEMM) nanoparticles. This toxicity for the free polymer may account for the lack of enhancement of the therapeutic index of PQ: the LD_{50} evaluated for PQ-loaded poly(DEMM) nanoparticles could be an intermediate value, falling between the intrinsic toxicity of free polymer and the decreased toxicity of bound PQ. Indeed, PQ entrapment in nanoparticles might attenuate its toxicity as reported for liposome-encapsulated PQ (Pirson et al., 1980) and for macromolecularly linked PQ, designed by Hofsteenge et al. (1986), however, the extent of the lowering of the toxicity also depends on the inherent toxicity of the polymer employed. This may account for the discrepancy between our observations and previously reported data for drug-loaded poly(alkylcyanoacrylate) nanoparticles whose toxicity is low (Couvreur et al., 1982).

The considerable lethal effect of nanoparticles on intravenous injection led us to administer the drugs to mice intraperitoneally. The blood schizonticidal effects observed could be biased, since the therapeutic scheme used (inoculum size, optimal time of drug administration, drug bioavailability, route of administration, etc.) is of great importance with regard to drug-specific action on several stages of the malaria (Fink, 1976). In our study, PQ binding to nanoparticles is expected to release the drug slowly; this accounts for the finding that the highest schizonticidal activity (ILS) was displayed when PQ-loaded poly (DEMM) nanoparticles were administered to mice 72 h or, preferably, 24 h prior to the infection of the animals. Both free and bound PQ confer a higher degree of partial cure when given to mice 24 h before inoculation with the parasites. This could mean that in our model such a delay is needed for the drug to reach optimal systemic availability when given intraperitoneally. Furthermore, our results show, in accordance with earlier findings (Pirson et al., 1980; Trouet et al., 1981) that the treatment of the mice 3 h before schizont inoculation does not markedly enhance their survival whereas injection of the drug 3 h post-inoculation appears to be more efficacious.

The nature of fixation of PQ on the polymer either through chemical bonding or merely via physical adsorption was not defined in our study. Desorption data are strongly suggestive of a quasi-irreversible binding mechanism and lend support to the hypothesis of covalent binding. Indeed, the nucleophilic secondary amine of PQ might participate as a reactant and catalyst in the anionic polymerization of DEMM monomers as has been shown earlier for water in the absence of more nucleophilic species (Donnely et al., 1977). The biological implications of such a PQ-DEMM conjugate are unpredictable. On the other hand, the antimalarial activity observed, which approaches that of free PQ, is likely to support the physicochemical nature of the PQ-nanoparticle link.

Conclusion

Owing to the versatility of its properties, namely, ease of synthesis, availability in large quantities, chemical stability, suitability to formulation as nanoparticles, etc., poly(DEMM) constitutes a valuable polymeric tool as a potential drug carrier. We formulated PQ-loaded nanoparticles as an alternative approach to increase the therapeutic index of PQ and its bioavailability for the liver. The characteristics of PQ-nanoparticles (size, homogeneity, drug binding) are favourable.

Their schizonticidal activity is higher than that of PQ diphosphate, and longer lasting which encourages their use as a sustained release system for PQ delivery. However, their toxicity excludes intravenous administration although loading of PQ lowers this toxicity. On the other hand, it must be ascertained whether their bioelimination does occur prior to use as systemic PQ vectors. The search for synthetic analogs of DEMM in order to design less toxic and biodegradable congeners is currently in progress.

Acknowledgements

We acknowledge the technical support of Miss I. de Zurpele and Mr J. Adline. One of the authors (T.K.M.M.) is indebted to the Association des Universités Partiellement ou Entièrement de Langue Française AUPELF/UREF for a grant.

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