Biochemical Pharmacology, Vol. 31, No. 21, pp. 3501–3507, 1982.
Printed in Great Britain.

0006-2952/82/213501–07 $03.00/0

THE DISPOSITION OF FREE AND LIPOSOMALLY ENCAPSULATED ANTIMALARIAL PRIMAQUINE IN MICE*

PHILIPPE PIRSON, ROLF STEIGER† and ANDRÉ TROUET
International Institute of Cellular and Molecular Pathology, 74, avenue Hippocrate, 1200 Brussels, Belgium

(*Received* 14 *December* 1981; *accepted* 26 *April* 1982)

Abstract—Plasma clearance, urinary excretion and tissue distribution of radiolabeled free (FPQ) and liposome-entrapped Primaquine (LPQ) in mice were monitored for 2 hr following intravenous administration. FPQ is eliminated very rapidly from the plasma and excreted predominantly in the urine, probably largely in a metabolized form. In decreasing order of magnitude, pronounced accumulation of label occurs in the liver, kidneys, lungs and skeletal muscle. Less than 1 per cent of the total initial dose is recovered in other tissues. Partial erythrocytic sequestration results in drug levels higher and more persistent in blood cells than in the plasma. Compared to the free drug form, Primaquine entrapped within negatively charged liposomes of the cholesterol-rich multilamellar type exhibits a prolonged plasmatic half-life and, within the observation period, excretion is 8-fold reduced. Liver accumulation of label is doubled, accounting for close to 50% of the injected dose; splenic uptake is tripled, while accumulation in the lungs, kidneys, heart and brain is drastically reduced. These differences in pharmacodynamic behaviour may explain why liposomal entrapment leads to diminished acute Primaquine toxicity.

Malaria, one of the most important human diseases of the tropics and subtropics, remains a crucial endemic problem world-wide.

Since World War II, a series of chemical classes of compounds have been synthesized as potential tissue schizontocidal drugs for the treatment of relapsing infections. From the 8-aminoquinolines, Primaquine (PQ) rapidly emerged as the most satisfactory candidate [1]. It is still the drug of choice for radical treatment, i.e. complete eradication of the parasites and prevention of relapses by destruction of the exoerythrocytic liver stages of *Plasmodium vivax* and *P. ovale*. Yet, wider use of Primaquine in prophylaxis and therapy is hampered by toxic side-effects, especially in individuals with glucose-6-phosphate dehydrogenase-deficient erythrocytes [2]. However, the rise of *Plasmodium falciparum* strains resistant to conventional blood schizontocides, such as Chloroquine [3], has revived considerable interest in PQ and its congeners. Surprisingly, relatively little is known about the pharmacology and metabolism of 8-aminoquinolines. Earlier studies on Pamaquine [4–6], Pentaquine [7, 8] and PQ [9] indicated rapid elimination of these compounds from the body following extensive metabolization. Thus, Price Evans *et al.* [10] found only relatively low concentrations of parent PQ in human blood and urine and ascribed this observation to rapid tissue sequestration and/or biotransformation of the drug involving at least four or five putative metabolites. Strother *et al.* [11] identified 5- and 6-hydroxy-PQ in dog urine, as well as a compound akin to a 5,6-dihydroxy derivative, after *in vitro* incubation of PQ with mouse liver enzyme extracts. Baty *et al.* [12] demonstrated the presence of the 6-methoxyquinoline nucleus in human blood.

Recently, liposomes have gained importance as experimental carriers of drugs [13], including PQ [14, 15]. Liposomal entrapment thereby modifies the tissue distribution pattern as well as, mostly favourably, the toxic properties of a given chemotherapeutic agent. Upon intravenous injection, drug-containing liposomes are rapidly removed from the blood and taken up to a large extent by endocytotic reticulo-endothelial system-derived elements of the liver and spleen, such as Kupffer cells and macrophages [16–19].

A previous investigation produced evidence that liposome-encapsulation of PQ results in reduced acute drug toxicity with unaltered therapeutic efficacy against liver stages of murine malaria [14].

The present study aims at elucidating the kinetic basis of this observation, namely whether, in comparison with free drug, encapsulation of PQ in liposomes leads to a higher degree of targeting *in vivo*.

MATERIALS AND METHODS

Phospholipids and drug. Phosphatidylserine (PS) (bovine brain, mol. wt = 810) and phosphatidylcholine (PC) (egg yolk lecithin, mol. wt = 785), chromatographically pure (grade 1), were obtained from

* This investigation received the financial support of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

† Present address: CIBA-GEIGY Ltd, AG 9.52, P.O. Box, CH 4002 Basel, Switzerland.

Lipid Products Ltd. (South Nutfield, Surrey, U.K.), and cholesterol (CH) (99% pure, mol. wt = 387) from Sigma Chemical Co. (St. Louis, MO).

Primaquine diphosphate (PQ) (Gold Label, mol. wt = 455.35) was purchased from Aldrich Chemical Co. (Milwaukee, WI).

Labeled products. [^{14}C]-Labeled dipalmitoyl phosphatidylcholine (DPPC), with a specific activity of 50 mCi/mmole, was obtained from Applied Science Laboratories (State College, PA). [^{3}H]-Labeled Primaquine diphosphate, with a specific radioactivity of 8.6 mCi/mg, was prepared by catalytic exchange of tritium in aqueous media by Amersham/Searle (Arlington Heights, IL). The crude product was kept frozen under nitrogen atmosphere and was purified before use by thin layer chromatography on silica gel [20].

Preparation of labeled liposomes. Liposomes containing PQ (LPQ) were prepared and processed essentially according to Bangham *et al.* [21]. An aqueous solution of [^{3}H]PQ (25 mg PQ/ml) in 0.015 M PBS (pH 4.5) was added to the dried film of lipids in a 50 ml glass bottle. The initial drug/lipid ratio in the preparation was 0.4 mg PQ/μmole total lipids equal to 25 mg PQ/62.5 μmole lipids/ml. The molar ratio of the lipids used was 1PS:4PC:5CH. [^{14}C]DPPC served as phospholipid marker (50 nCi/μmole of total lipid preparation) at a molar fraction of labeled to unlabeled lipid of less than 1%. The lipid film was resuspended under nitrogen by vortexing for 10 min at 37°. After sonication for 10 × 30 sec in a Branson B12 sonifier (Branson Sonic Power Co., Dambury, U.S.A.) (75W, 20 kHz, 27.5 mm probe), the vesicles were purified by elution with cold 0.15 M PBS (pH 7.2) through Sepharose 4B. Only the fraction corresponding to the void volume of the column containing multilamellar vesicles was collected. The degree of drug encapsulation and the specific concentration of PQ were measured by: (a) absorbance of PQ at 360 in a Gilford 250 Spectrophotometer using aliquots of fresh preparations dissolved in a 1:1 mixture of DMSO–*n*-butanol read against freshly prepared standards, and (b) respective determination of cholesterol and phospholipids with the enzymatic CHOD-PAP and colorimetric phospholipid test kits (Boehringer, Mannheim, F.R.G.).

The incorporation rate was 97 ± 11 (S.D.) μg PQ/μmole total lipids. Under the above conditions, 13.4 ± 1.9% of the total drug in the aqueous phase was entrapped within the lipid material.

Plasma elimination kinetics. [^{3}H]PQ (4.2 μCi/mg) and [^{3}H]PQ in [^{14}C]DPPC-labeled liposomes (3.2 μCi/mg PQ and 50 nCi/μmole total lipids) were injected i.v. via a tail vein into TB_{ESP} male mice. The administered dose was 0.25 mg PQ and 2.44 ± 0.19 μmole of lipids per 25 g mouse given i.v. in a volume of 0.4 ml. The regimen was adjusted precisely to the individual animal weights to obtain a homogenous initial plasmatic concentration of PQ.

At different time intervals (1, 5, 10, 20, 40, 60 and 120 min post-injection), the blood was collected from the femoral vein on EDTA as anticoagulant and centrifuged for 2 min at 4° in a Beckman Microfuge B (Beckman Spinco Division, Palo Alto, CA). Radioactivity was measured in a Packard LSC Scintillation Counter (Packard, Downers Grove, IL) after adding 0.1 ml of plasma to 10 ml of Lumagel (Lumac AG, Basel, Switzerland). The counts were expressed as dpm/ml after correcting for quenching, converted into μg or nmole PQ equivalents and nmole total lipids and, finally, into percentages of administered dose.

The kinetic parameters were computed from the above plasma concentrations in the 1–120 min interval, using a two-compartment iterative model [22].

Blood, urine and tissue distribution. In separate experiments under the above conditions, drug and liposome-derived lipids were determined in the liver, spleen, brain, heart, lungs, kidneys, stomach, duodenum, rectum and muscles, as well as in the blood and urine.

At each time point, six animals were decapitated; their organs, blood and urine were collected immediately and kept at 0°. The tissues were taken up in 1–2 ml cold saline and homogenized in a Potter–Elvehjem glass homogenizer with a tightly fixed Teflon pestle.

After rinsing with an equal volume of fresh saline, the pooled suspensions were sonicated for 30 sec at 50 W. Blood was collected on EDTA as anticoagulant and sonicated similarly.

Aliquots of tissue homogenates, blood and urine samples (100 μl or 250–500 μl for tissues showing low uptake) were digested at 60° in 1 ml of a Lumasolve–Isopropanol mixture (10:3), discoloured for 15 min by the addition of 0.2 ml H_2O_2 (0.5 ml for blood and urine) and finally added to 10 ml Lumagel–1N HCl (10:1) (15 ml for blood). Lumasolve and Lumagel were obtained from Lumac AG, Basel. Radioactivity was measured in a Packard liquid scintillation counter and corrected for quenching. Tissue and blood protein were measured by the fluorescamine method [23]. The gross values for tissue concentrations of PQ, lipids and protein were corrected for blood contaminating the tissue samples by assaying immunologically for serum albumin according to Mancini *et al.* [24].

The corrected values are expressed as pmole PQ equivalents and pmole lipids per mg protein. The total amounts per organ are given in percentages of administered dose.

In a separate experiment, groups of six mice were injected *in vivo* with [^{14}C]-labeled PQ in the nucleus (quinoline-2,4-^{14}C diphosphate, 1.55 mCi/mmol; New England Nuclear, Boston, MA) at a concentration of 10 mg/kg (1 μCi/mg). At different times after injection (between 5 min and 4 hr), blood and plasma are collected from the same animal and the haematocrit was determined in a microhaematocrit MC centrifuge (Damon, I.E.C., Needham Heights, MA). The radioactivity was checked on aliquots of blood and plasma treated in the same condition and as described before. The drug concentration is expressed as μg PQ equivalent per ml of blood and volume of plasma (based on the respective haematocrit value for each blood sample).

In vitro *study of Primaquine release from liposomes.* A volume of 0.2 ml of the doubly-labeled LPQ, prepared as described above, was added to three tubes containing, respectively, 4.8 ml of buffer (PBS), fresh mouse blood on EDTA as anticoagulant

and serum (50 and 100%) to obtain a final concentration of 2 μmole lipids/ml. At different times of incubation at 37°, between 0 and 5 hr, duplicate samples (0.250 ml) were taken and directly cooled at 0°. The blood samples were centrifuged 1 min in a Beckman microfuge B to remove the red blood cells. The released PQ was separated from that remaining into liposomes by centrifuging the samples on a modified Sephadex G25 M column, as described by Layton and Trouet [25]. By this method, the free drug was entirely retained on the column, while more than 95% (97.1 ± 1.3%; S.D., $n = 168$) of the original lipid added was recovered in the liposome eluant. Lipids and Primaquine concentrations were assessed by the radioactivity after adding 0.1 ml of eluant to 10 ml of Lumagel. The results of three separated experiments were expressed as percentages of the initial PQ load at time O.

RESULTS

In vitro *release of Primaquine from liposomes*

When liposomal Primaquine was incubated *in vitro* for a period of 5 hr in the presence of whole mouse blood, 20% of the PQ originally present was released. This loss of PQ predominantly occurred during the first 30 min of contact with the blood (Fig. 1). When total blood was replaced by 100% serum, only 54% of the PQ was retained by the liposomes, whereas with 50% serum results quite similar to that obtained for whole blood were found. In buffer alone the liposomes remained intact, and lost PQ progressively and linearly with time, a reflection of a constant diffusion phenomenon.

Pharmacokinetics and urinary excretion of total radioactivity after injection of free and liposomal Primaquine

The total plasma radioactivity after a single i.v. injection of labeled free PQ (FPQ) and doubly-labeled Primaquine-liposomes (LPQ) are depicted in Fig. 2. The respective values are expressed as the percentage of the administered dose.

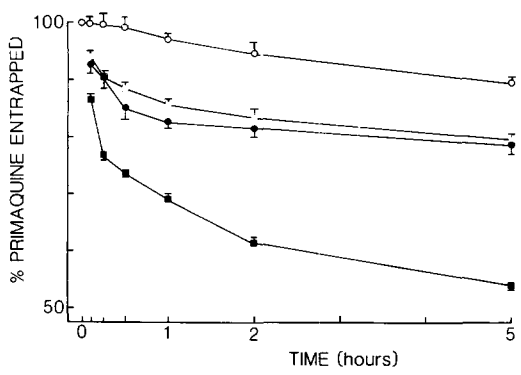

Fig. 1. Release of [^{3}H]Primaquine from liposomes induced by incubation at 37° in different media. The final concentration of the PQ–liposome preparation is 2 μmole lipids and 190 μg PQ per ml of incubating mixture. The results (means ± S.E., $n = 3$) represent the amount of PQ remaining entrapped inside liposomes expressed as per cent of the initial PQ concentration. (○——○) Phosphate buffer; (●——●) whole mouse blood with 10 μl EDTA; (□——□) 50% and (■——■) 96% final mouse serum.

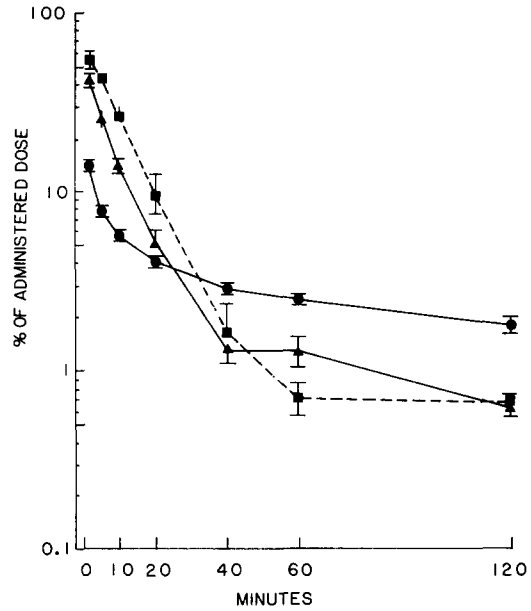

Fig. 2. Plasma levels of [^{3}H]Primaquine and [^{14}C]DPPC-labeled lipids after intravenous administration (means ± S.E., $n = 9$). ●——● Free and ▲——▲ liposomal PQ equivalent (10 mg/kg); ■——■ liposomal lipids (97.2 μmole/kg).

One minute after administration of FPQ, less than 15% of the initial load is detected in the plasma. The remaining label is then eliminated slowly with a $T^{\frac{1}{2}}$ of 105 min.

When the drug is administered as LQP, drug disappearance is slower, reaching 42% of its initial concentration within the first minute ($T^{\frac{1}{2}} \sim 5.2$ min), whilst the remaining label disappears faster than free drug ($T^{\frac{1}{2}} \sim 67$ min). The LPQ-derived lipid label behaves similarly, involving 45% of the lipids. After that, the liposomes are eliminated more slowly ($T^{\frac{1}{2}} \sim 4.6$ hr). Elimination of LPQ is similar in the plasma and the blood (Fig. 4B). The difference between the respective PQ and labeled lipid constituents 1 min after the administration indicates that some 15% of the total PQ entrapped initially within the liposomes dissociate rapidly upon i.v. injection. Primaquine mixed with empty liposomes (not shown here) at concentrations equivalent to LPQ disappears exactly as free drug. For free and liposomal PQ respectively, the estimated apparent distribution volume is 19.4 and 7.0 ml, while the total clearance is 0.143 and 0.168 ml min^{-1}.

In individual animals, the FPQ concentration was consistently higher in the whole blood than in the plasma (volume based on the haematocrit value of each blood sample), as assessed by the Student's t-test for two-tailed hypotheses, $P[t] < 0.001$ between 5 and 180 min (Table 1). The discrepancy between the respective concentrations for each sampling point corresponds to 36.2 ± 1.3% (S.E., $n = 5$ points) within 120 min. The largest difference occurs at 180 min and accounted for 67% of the blood con-

Table 1. Respective blood and plasma concentrations of Primaquine-derived radioactivity

Time (min)	Primaquine concentration* (μg) in blood	in plasma	Difference† (%)
5	13.8 (0.15)	9.2 (0.14)	33.2
15	9.6 (0.11)	5.7 (0.28)	40.4
30	7.6 (0.16)	5.0 (0.22)	33.9
60	5.9 (0.09)	3.8 (0.13)	36.3
120	5.1 (0.05)	3.2 (0.21)	37.4
180	4.7 (0.51)	1.5 (0.38)	67.5
240	1.2 (0.04)	1.1 (0.09)	5.8

* The values (means ± S.E., $n = 6$) are expressed in μg of [^{14}C]Primaquine equivalents per ml of blood and per volume of plasma based on the haematocrit value corresponding to 1 ml of each blood sample.

† For each time, the difference between the respective blood and plasma concentrations of Primaquine equivalents is expressed as the percentage of the blood level and represents the amount of drug associated with the red blood cells.

centration, whilst the two respective levels become quite similar after 4 hr. It is interesting to note that the disappearance of the [^{14}C]-labeled and tritiated PQ are close together in the plasma and also in blood (elimination half-lives of 2.32 and 2.66 hr, respectively).

For FPQ, urinary excretion of total radioactivity peaks at 40 min post-injection whereas the LPQ-derived label in the drug has its maximum between 40 and 60 min. Liposome encapsulation lead to a 8-fold reduced PQ excretion peak. The label from the drug recovered in the urine within 2 hr after i.v. administration is equivalent to about 16% of total FPQ and to 2% of total entrapped PQ radioactivity injected.

Tissue distribution of free and liposomal Primaquine

Tissue concentrations of drug and lipid label 20 and 120 min after injection are summarized in Fig. 3. Their disposition in blood, liver and spleen over 120 min is depicted in Fig. 4. The amount of tritium recovered is assumed to be proportional to the level of Primaquine and/or of its putative metabolites.

Major sites of FPQ-derived radioactivity are the liver, lungs, kidneys and skeletal muscle. Primaquine-derived radioactivity thereby accumulates progressively in the liver until 30% of the dose is localized there 20 min after administration. Skeletal muscle accounts for more than 8% within the first 20 min but for only 1% of the initial dose at 120 min. Four to five per cent are found in the kidneys and lungs between 20 and 120 min. Not more than 1% is taken up by spleen, heart, brain, stomach, duodenum and rectum.

Alternatively, when PQ is entrapped in liposomes, the amount of drug-derived radioactivity 20 min after administration, is increased for liver (1.6×) and spleen (4×) and is equal to 50 and 4% of the total dose, respectively, whereas the levels found in kid-

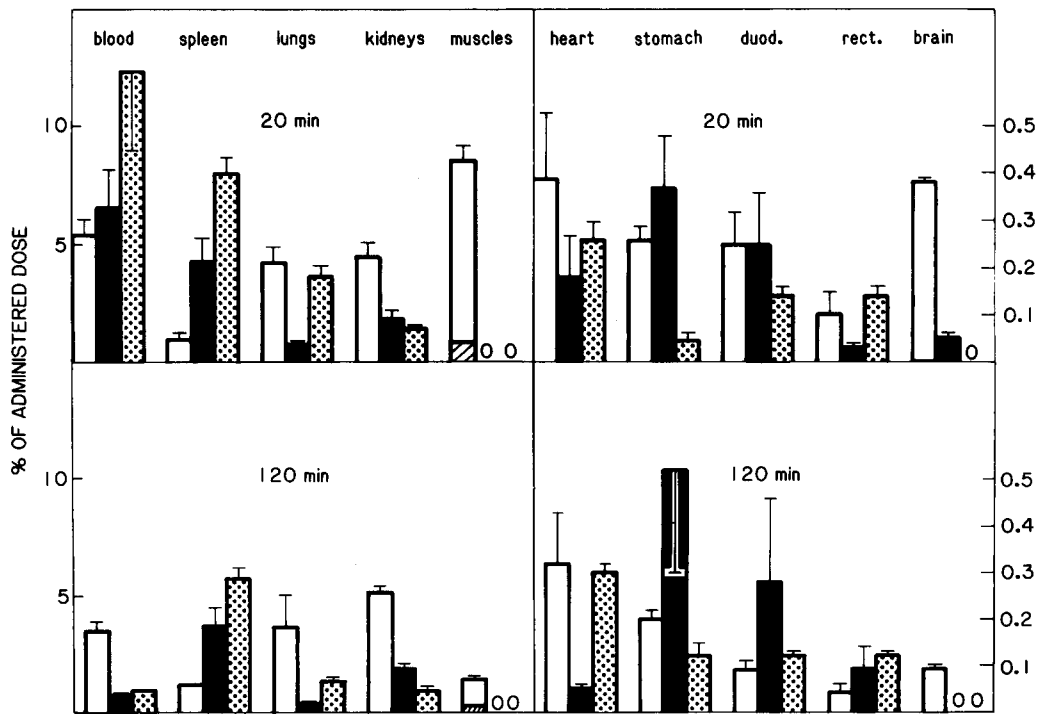

Fig. 3. Tissue distribution of free [^{3}H]PQ (white area) and liposome-entrapped Primaquine, [^{3}H]PQ (black area) + [^{14}C]DPPC-labeled lipids (dotted area), 20 and 120 min after i.v. injection. The histograms (means ± S.E. of 6 experiments) represent percentages of the initial drug dose (10 mg PQ + 101.2 μmole lipids/kg) recovered in the whole organs. Concentrations in muscles are expressed per g tissue (hatched). Values for total skeletal muscles are approximated, assuming that their mass corresponds to ~40% of the body weights. (0) No detectable radioactivity.

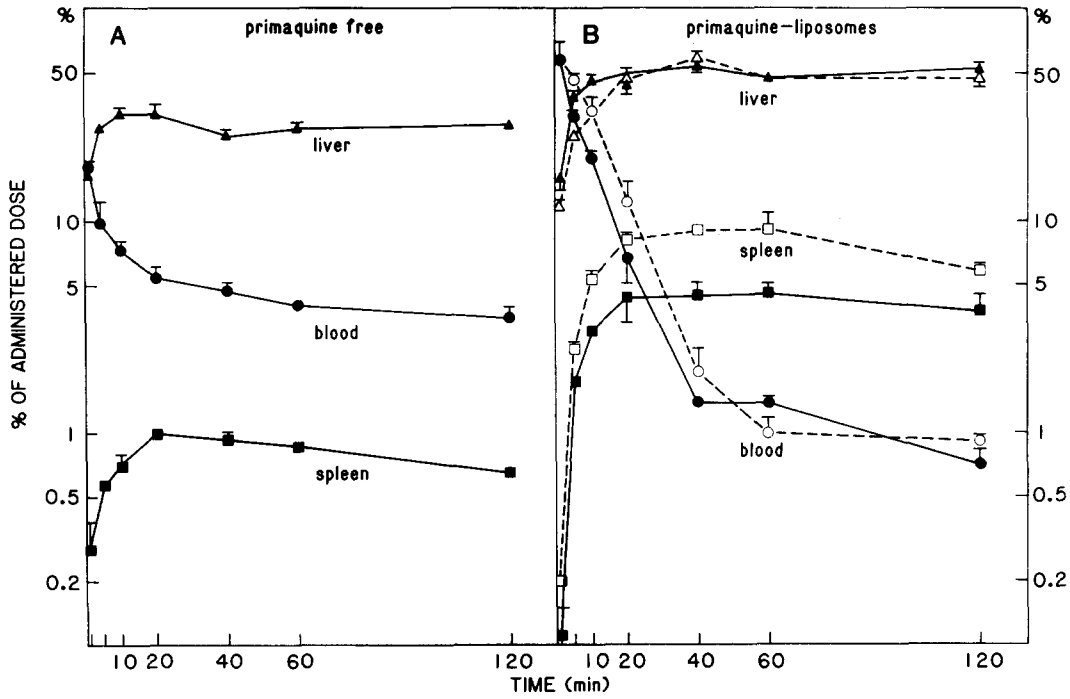

Fig. 4. Hepatic and splenic distributions of free and liposomal Primaquine, and their respective blood levels (means ± S.E., $n = 6$). (A) Free Primaquine; (B) Primaquine in liposomes: PQ (filled symbol) and lipids (open symbol).

Table 2. Concentration of [^{3}H]Primaquine equivalents and [^{14}C]DPPC-labeled liposomes in selected tissues after i.v. administration of free and entrapped drug

Tissues	Time*	Free Primaquine†	Liposomes-Primaquine Primaquine†	Liposomes-Primaquine Lipids‡
Liver	20	589 ± 113	907 ± 53	4.057 ± 702
	120	530 ± 62	882 ± 56	3.675 ± 436
Spleen	20	616 ± 65	1.960 ± 430	18.509 ± 3.670
	120	364 ± 26	1.400 ± 120	11.355 ± 1.840
Heart	20	257 ± 64	52 ± 29	596 ± 50
	120	286 ± 62	42 ± 19	945 ± 190
Kidney	20	382 ± 58	103 ± 21	364 ± 17
	120	523 ± 57	114 ± 9	258 ± 42
Lungs	20	3.706 ± 1.250	267 ± 36	6.139 ± 450
	120	3.538 ± 1.560	118 ± 24	2.217 ± 330
Stomach	20	63 ± 16	62 ± 16	51 ± 20
	120	37 ± 5	84 ± 31	122 ± 40
Duodenum	20	38 ± 9	38 ± 10	97 ± 20
	120	15 ± 3	54 ± 30	144 ± 50
Rectum	20	131 ± 79	22 ± 8	655 ± 270
	120	35 ± 24	45 ± 21	296 ± 50
Brain	20	23 ± 2	6	0§
	120	4 ± 1	4	0
Muscles	20	28 ± 2	0	0
	120	5 ± 1	0	0
Blood	20	68 ± 9	78 ± 13	657 ± 140
	120	44 ± 7	11 ± 2	57 ± 5

* Sampling time in minutes after i.v. administration of the drug.

† Results are expressed in pmole Primaquine equivalents/mg protein. Means ± S.E. of 6 experiments.

‡ Results are expressed in pmole total lipids/mg protein. Means ± S.E. of 6 experiments.

§ Not detectable.

ney, lungs and brain are drastically decreased. Only marginal radioactivity is present in muscle. The distribution of the drug administered as LPQ is largely comparable to that of the lipids, indicating that vesiculation modifies PQ distribution. Interestingly, the drug–lipid ratios are relatively low for the spleen and lungs.

The concentrations of PQ or lipids per mg protein represent tissue-specific uptake. Hence this parameter is a more reliable measure of the specificity of drug delivery. Figure 4 illustrates the specific drug levels in blood, liver and spleen over 120 min after i.v. injection of FPQ and LQP, and Table 4 shows the concentrations reached in all tissues after 20 and 120 min.

For FPQ the highest concentration is reached in the lungs (~3700 pmole/mg protein), followed by the liver and spleen (~600 pmole/mg protein). For the entrapped drug, the major uptake is found for the spleen (~1900 pmole/mg protein) and liver (~900 pmole/mg protein), while the concentrations in the other tissues are below 300 pmole/mg protein. The specific uptake pattern of liposomal lipids is essentially similar to that of encapsulated drug (~18 nmole/mg protein for the spleen and ~4 nmole/mg protein for liver), except for the lungs (~6 nmole/mg protein) where the lipid concentration is proportionally much higher. It can also be noted that the amount of vesicular lipids accumulating in the spleen, lungs and liver varies with time. At 20 min, the specific lipid concentration was higher in the spleen that in the lungs and liver. By 120 min, levels are considerably decreased for the lungs (3×) and spleen (1.5×), while remaining unchanged in the liver.

DISCUSSION

Fate of free Primaquine

This investigation shows that free Primaquine is removed very rapidly from the circulation, as has previously been described in the case of 8-aminoquinolines [4–12]. The half-life of elimination from blood and serum of labeled PQ reported here is close to those found for species other than the mouse [10, 11]. Our observations in Table 2 indicate that the elimination of PQ from plasma is more rapid than from total blood, and therefore suggests that FPQ might partially be taken up or bound by red blood cells. This sequestration occurs very rapidly upon injection and is in agreement with investigations by others on dog [11] and human [12] red cells.

The PQ-associated radioactivity, upon i.v. injection, accumulates rapidly in organs, mostly in liver, followed by the kidneys, lungs and skeletal muscle. A similar tissue distribution has been reported in the case of the rat [26], where also the highest concentration of drug per g of tissue was found in the lungs. Major uptake by liver and lungs was already displayed by Pamaquine [4, 5] and derivatives [4]. This property seems to be common to all 8-aminoquinolines. It is possible that the organs which preferentially take up PQ label do so by free diffusion of the drug from the body fluids, since they have a large blood flow capacity.

Primaquine acts predominantly against plasmodial exoerythrocytic stages in liver. The high hepatic levels of PQ would be consistent with the drug efficiency, since the bulk of PQ sequestered by rat liver [27] is localized in parenchymal cells when malaria parasites develop as schizonts.

The catabolic state of the compound has not been determined in this study, and total radioactivity therefore represents both unchanged and metabolized PQ, since a rapid and extensive biotransformation of Primaquine has been demonstrated [26, 28, 29]. Urine excretion of labeled compound appears early and is important not only in mice, but also in the rat [29] and dog [11].

Fate of liposomal Primaquine

The most important effect of encapsulation is a complete change in the distribution pattern of the antimalarial drug in mice, with an enhanced accumulation of PQ-radioactivity in reticulo-endothelial cell-rich tissues, such as liver and spleen, while the amount of label is drastically reduced in other tissues, such as lungs, kidney, heart and brain. This is in agreement with data from other authors [16, 17], showing a selective uptake of liposomes by liver and spleen, predominantly captured by the reticuloendothelial cells [18, 19, 30]. The specific tissue concentration of LPQ is consistently higher in the spleen than in the liver. This observation, also characteristic of other drug–liposome preparations [31, 32], reflects the high phagocytic activity of the spleen involved in the elimination of vesicles from the bloodstream.

The negatively charged multilamellar liposomes (MLV) which are used in this study are known to be rapidly removed from the circulation [19, 33, 34]. These vesicles partially modify the fate of Primaquine in mouse plasma but are not able to prolong the blood retention of the drug. The radioactivity of LPQ after i.v. injection falls more slowly, while its elimination is more rapid. Also the total level of LPQ present during the 2-hr period is less than that of FPQ, as reflected by the area under the plasma concentration tissue curve (Fig. 2).

The urine excretion of label is highly reduced within the same time period, as previously reported for liposomal antitumoral agents, by their reduced renal clearance as well as metabolic degradations [31, 35].

Incorporated PQ partially dissociates from the liposomes in blood both *in vitro* (Fig. 1) and *in vivo* (Fig. 2), but the loss of PQ appears to be faster upon injection, as noted elsewhere [36]. The stability of our PQ-liposomes greatly depends on the serum concentration (Fig. 1), as also reported by Gregoriadis and Davis [36]. The cholesterol increases the stability of liposomes and reduces considerably the bilayer permeability towards solutes by preventing phospholipid loss to high density lipoproteins present in serum, both *in vitro* [37, 38] and *in vivo* [30, 36]. This effect is a function of the cholesterol concentration and is optimal at 50 mole % of cholesterol as in our case. Our results on the blood stability *in vitro* and the improvement of the tissue distribution of LPQ, as well as the comparable disposition of PQ

and lipid labels from LPQ, indicate that the bulk of PQ is transported in intact liposomes.

In conclusion, the main consequence of incorporating PQ into liposomes is a reduced access of the drug to non-target tissues, such as the lungs, heart, kidneys and brain, which is the primary cause of subacute PQ toxicity in animals [39]. Thus more specific homing may be the basis for a 3.5-fold diminished acute toxicity observed earlier, which enables a single dose curative treatment [14]. Further studies should aim at identifying the metabolites of [^{3}H]-labeled PQ to enable our data to be related to the toxic and therapeutic effects of free and liposomal drug.

Acknowledgements—We wish to thank Professor C. de Duve for his encouraging interest, Drs E. Mayhew and D. Layton for helpful suggestions related to the preparation and application of liposomes, and Mrs S. Cornelis-Marcelis for her excellent technical assistance. We are equally indebted to Dr D. Deprez-De Campeneere and R. Baurain for valuable experimental advice.

REFERENCES

1. J. H. Edgcomb, J. Arnold, E. H. Yount, A. S. Alving and L. Eichelberger, *J. natn. Malaria Soc.* **9**, 285 (1950).
2. A. R. Tarlov, G. J. Brewer, P. E. Carson and A. S. Alving, *Arch. intern. Med.* **109**, 209 (1962).
3. WHO, Geneva, Technical Report Series No. 529 (1973).
4. L. H. Schmidt, in *Survey of Antimalarial Drugs* (Ed. J. W. Edwards), Vol. 1, p. 106. Ann Arbor, Michigan (1946).
5. C. G. Zubrod, T. J. Kennedy and J. A. Shannon, *J. clin. Invest.* **27**, 114 (1948).
6. H. L. Bami, M. S. Dhatt, G. S. Ahluwalia and A. P. Ray, *Ind. J. Malariology* **14**, 1 (1960).
7. R. C. Elderfield and L. L. Smith, *J. Am. chem. Soc.* **75**, 1022 (1953).
8. C. C. Smith, *J. Pharmac.* **116**, 67 (1956).
9. J. Arnold, A. S. Alving, R. S. Hockwald, C. B. Clayman, R. J. Dern, E. Beutler, C. L. Flanagan and G. M. Jeffery, *J. lab. clin. Med.* **46**, 391 (1955).
10. D. A. Price Evans, H. M. Gilles, J. Greaves, K. A. Fletcher, D. Bunnag and T. Harinasuto, *WHO Bull.* **59**, 407 (1981).
11. A. Strother, I. M. Fraser, R. Allahyari and B. E. Tilton, *WHO Bull.* **59**, 413 (1981).
12. J. D. Baty, D. A. Price and P. A. Robinson, *Biomed. Mass Spectrometry* **2**, 304 (1975).
13. G. Gregoriadis, in *Liposomes in Biological Systems* (Eds G. Gregoriadis and A. C. Allison), p. 25. John Wiley, Chichester (1980).
14. P. Pirson, R. F. Steiger, A. Trouet, J. Gillet and F. Herman, *Ann. trop. med. Parasitol.* **74**, 383 (1980).
15. C. R. Alving, E. A. Steck, W. L. Chapman, V. B. Waits, L. D. Hendricks, G. M. Swartz and W. L. Hanson, *Life Sci.* **26**, 2231 (1980).
16. G. Gregoriadis and B. E. Ryman, *Biochem. J.* **129**, 123 (1972).
17. Y.-E. Rahman, M. W. Rosenthal, E. A. Cerny and E. S. Moretti, *J. lab. clin. Med.* **83**, 640 (1974).
18. A. W. Segal, E. J. Wills, J. E. Richmond, G. Slavin, C. D. V. Black and G. Gregoriadis, *Br. J. exp. Path.* **55**, 320 (1974).
19. F. H. Roerdink, E. Wisse, H. W. M. Morselt, J. Van der Meulen and G. L. Scherphof, in *Küpffer Cells and Other Liver Sinusoidal Cells* (Eds E. Wisse and D. L. Knook), p. 263. Elsevier/North-Holland Biomedical Press, Amsterdam (1977).
20. S. A. Fusari, I. J. Holcomb and R. B. Luers, *J. chromatogr. Sci.* **13**, 589 (1975).
21. A. D. Bangham, M. W. Hill and N. G. A. Miller, in *Methods in Membrane Biology* (Ed. E. D. Korn), Vol. 1, p. 1. Plenum Press, New York (1974).
22. C. M. Metzler, in *A Users Manual for Nonlin and Associated Programmes*. Upjohn Company, Kalamazoo (1974).
23. S. Stein, P. Bohlen, J. Stone, W. Dairman and S. Udenfriend, *Archs Biochem. Biophys.* **155**, 203 (1973).
24. G. Mancini, A. D. Carbonara and J. F. Heremans, in *Immunochemistry*, Vol. II, p. 235, Pergamon Press, Oxford (1965).
25. D. Layton and A. Trouet, *Eur. J. Cancer*, **16**, 945 (1980).
26. D. J. Holbrook, J. B. Griffin, L. Fowler and B. R. Gibson, *Pharmacology* **22**, 330 (1981).
27. J. E. Smith, P. Pirson and R. E. Sinden, *Ann. trop. Med. Parasitol.* **76** (1982).
28. P. E. Carson, R. Hohl, M. V. Nora, G. W. Parkhurst, T. Ahmad, S. Scanlan and H. Frischer, *WHO Bull.* **59**, 427 (1981).
29. F. H. Ryer, *Fedn Proc.* **30**, 335 (1971).
30. G. Scherphof, F. Roerdink, D. Hoekstra, J. Zborowski and E. Wisse, in *Liposomes in Biological Systems* (Eds G. Gregoriadis and A. C. Allison), p. 179. John Wiley, Chichester (1980).
31. H. K. Kimelberg, *Biochem. biophys. Acta* **448**, 531 (1976).
32. G. Gregoriadis, D. E. Neerunjun and R. Hunt, *Life Sci.* **21**, 357 (1977).
33. R. L. Juliano and D. Stamp, *Biochem. biophys. Res. Commun.* **63**, 651 (1975).
34. M. R. Mauk and R. C. Gamble, *Proc. natn. Acad. Sci. U.S.A.* **76**, 765 (1979).
35. R. L. Juliano and D. Stamp, *Biochem. Pharmac.* **27**, 21 (1978).
36. G. Gregoriadis and C. Davis, *Biochem. biophys. Res. Commun.* **89**, 1287 (1979).
37. M. C. Finkelstein and G. Weissmann, *Biochem. biophys. Acta* **587**, 202 (1979).
38. E. Mayhew, Y. M. Rustum, F. Szoka and D. Papahadjopoulos, *Cancer Treat. Res.* **63**, 1923 (1979).
39. C. C. Lee, L. D. Kinter and M. H. Heiffer, *WHO Bull.* **59**, 439 (1981).