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Biodegradable microspheres: XIV. Effect of microparticle-bound primaquine on *L. donovani* in mice

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Summary

The antileishmanial effect of microparticle-bound primaquine (PQ) was studied in mice. PQ was coupled to polyacryl starch microparticles (PSM), a lysosomotropic drug carrier, via a biodegradable tetrapeptide spacer arm. The microparticle-bound PQ was more than 100 times more efficient than free drug in reducing parasite load. Empty PSM had no effect. The increased efficiency was explained by the targeting of the PQ-loaded PSM mainly to the liver in the infected animals. Also, the tissue distribution and elimination of PQ-PSM was studied.

Introduction

The leishmaniases are a group of diseases caused by different species of protozoan parasites of the genus *Leishmania*. After infection, the parasites survive and multiply in the RES of the host. The parasites are spread over all continents, except Australia, and about 350 million people are at risk of infection (WHO, 1990). An incidence rate of 400 000 new cases per year has been reported (UNDP/World Bank/WHO, 1980). The prevalence is believed to be about 12 million cases (WHO, 1990) and the leishmaniases are one of the six diseases given priority to by the WHO (1984) The most severe form, visceral leishmaniasis, caused by *L. donovani,* is almost invariably fatal if not treated (Rees and Kager, 1987).

Leishmaniasis is generally treated with pentavalent antimonials, which have high potential toxicity (Steck, 1974), and is not always effective (Bryceson et al., 1985). No vaccine is currently available. To improve therapy by the selective delivery of antiparasitic drugs to the RES, different particulate drug carriers have been tried, especially liposomes (reviewed by Alving, 1986).

Polyacryl starch microparticles (PSM) (reviewed by Edman et al., 1987) is another microparticulate drug carrier that has shown promising results in experimental antileishmanial therapy, both in vitro (Stjärnkvist et al., 1987) and

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in vivo (Baillie et al., 1987). After intravenous administration, the microparticles are taken up by the RES, mainly in the liver (Laakso et al., 1986). Drugs can be covalently attached to PSM via a peptide spacer arm, in a way allowing drug release in the lysosomes but not in serum (Laakso et al., 1987).

PQ is traditionally used as an antimalarial, but also has antileishmanial activity in hamsters (Kinnamon et al., 1978) and mice (Peters et al., 1980). In these studies PQ has about twice the antileishmanial activity of that of the pentavalent antimonials. The use of PQ is, however, hampered by its toxicity to red blood cells causing hemolytic anemia, especially in individuals with glucose 6-phosphate dehydrogenase deficiency (Tarlov et al., 1962). Intravenously administered to mice, PQ is rapidly cleared from the circulation, and after 20 min, 30% of the dose is in the liver (Pirson et al., 1982). Moreover, as shown in isolated rat liver, the bulk of PQ taken up by the liver is localized in parenchymal cells (Smith et al., 1983).

In the present study, a tetrapeptide derivative of PQ was synthesized and coupled to PSM and the antileishmanial effect of free and microparticle bound PQ was assessed in mice. Also, the organ distribution and elimination of PQ-loaded ^{14}C-labelled PSM were studied.

Materials and Methods

Chemicals

N-tert-Butyloxycarbonyl-L-leucine, L-alanine benzyl ester *p*-tosylate and *N-tert*-butyloxycarbonyl-L-alanine were from Bachem Feinchemikalien AG (Bubendorf, Switzerland). *N*-Hydroxysuccinimide was from Fluka AG (Buchs, Switzerland). *N,N,N',N'*-Tetramethylenediamine (TEMED), 1,1-carbonyldiimidazole (CDI) and dicyclohexylcarbodiimide (DCC) were from Merck (Darmstadt, Germany). Primaquine diphosphate and *N,N*-dimethyloctylamine were from Janssen Chimica (Beerse, Belgium). The soluble starch used for the microparticle preparation (MD6) was a gift from Dr Lars Svensson, Stadex AB (Malmö, Sweden).

Analytical thin-layer chromatography

Analytical TLC was performed using Merck silica gel 60 F-254 glass-backed plates. Spots were visualized by UV or ninhydrin spray. The following mobile phases were used: A, methanol : chloroform : acetic acid (45 : 5 : 2); B, *n*-butanol : acetic acid : water (4 : 1 : 1).

High-performance liquid chromatography

The HPLC analyses were carried out with an HP 1090 liquid chromatograph with a 3392A integrator (Hewlett Packard). The column used was a Spherisorb S5 ODS2 (250 × 4.6 mm; Phase Separations Inc.). The mobile phase consisted of 0.025 M NaH_2PO_4 (pH 3), 0.60 μM *N,N*-dimethyloctylamine, and 30% acetonitrile. The flow rate was 1.0 ml/min and the wavelength 254 nm.

Particle size determination

The microparticle size was measured with a Coulter counter model T_A II (Coulter Electronics Ltd, U.K.) equipped with a 50 μm aperture tube, detecting particles from 1 to 20 μm. About 98% of the unmodified particles had a diameter of < 2.5 μm. After coupling with Ala-Leu-Ala-Leu-PQ and glycine, 94 and 97%, respectively, had a diameter of < 2.5 μm.

Synthesis of peptides

N-(tert-Butyloxycarbonyl)-L-leucylprimaquine (Boc-Leu-PQ; 1) *N-tert*-Butyloxycarbonyl-L-leucine (Boc-Leu) (5.00 g, 20.06 mmol) and carbonyldiimidazole (CDI) (3.20 g, 20.00 mmol) were dissolved in 40 ml of dry DMF. The mixture was stirred under a nitrogen atmosphere at 0°C for 30 min and at room temperature for 30 min. Primaquine diphosphate (PQ) (12.01 g, 26.37 mmol) was dissolved in 250 ml of water and converted to base by adding 1 M NaOH to give a pH of about 11. The PQ base was extracted with ether and the combined extracts were dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The resulting oil was dissolved in 20 ml of dry dimethylformamide (DMF), added to the CDI/Boc-Leu mixture, and stirred under nitrogen for 1 h at 0°C and overnight at room temperature. The DMF was evaporated under reduced pressure and the oily residue dissolved in 100 ml

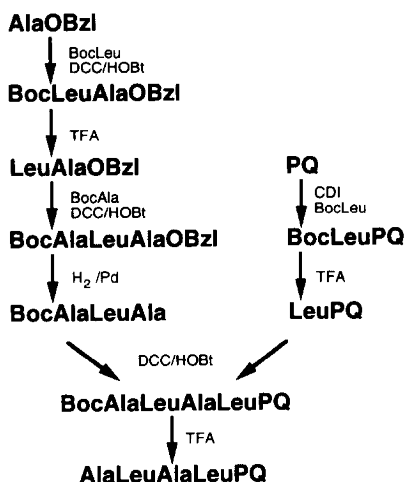

Fig. 1. Pathways for the peptide syntheses.

ether, washed with 0.1 M $NaHCO_3$ pH 9 and 0.1 NaH_2PO_4 pH 6, dried over Na_2SO_4 and evaporated under reduced pressure. The resulting yellow solid was purified by flash chromatography (Still et al., 1978) on a silica gel column with diethyl ether as mobile phase. The product crystallized after drying (Na_2SO_4) and evaporation of the ether. Yield: 6.21 g (65.6%); R_f(A): 0.78.

L-Leucylprimaquine (Leu-PQ; 2) Compound **1** (2.00 g, 4.23 mmol) was dissolved in 25 ml of trifluoroacetic acid (TFA) and stirred for 1 h at room temperature. The excess reagent was removed by evaporation under reduced pressure. The resulting oil was mixed with 50 ml of 1 M $NaHCO_3$ and the product was extracted with ether. The combined organic phases were dried over Na_2SO_4 and evaporated, giving the product as yellow crystals. Yield: 1.52 g (96.4%); R_f(B): 0.61.

N-(tert-Butyloxycarbonyl)-L-leucyl-L-alanine benzyl ester (Boc-Leu-Ala-OBzl; 3) L-Alanine benzyl ester *p*-tosylate (14.10 g, 40.12 mmol) was dissolved in 40 ml of dry DMF at 0°C, and triethylamine (5.56 ml, 40.12 mmol) was slowly added. Boc-Leu (10.00 g, 40.11 mmol) and hydroxybenzoetriazole (HOBt) (12.27 g, 80.22 mmol) were dissolved in 30 ml of dry DMF, and dicyclohexylcarbodiimide (DCC) (9.10 g, 44.10 mmol) dissolved in 10 ml of dry DMF was added dropwise. The mixture was stirred under a nitrogen atmosphere for 3 h at 0°C and overnight at room temperature. The urea was filtered off and the solvent was evaporated under reduced pressure. The oily residue was dissolved in 100 ml of ethyl acetate, washed with 1 M citric acid, brine, 1 M $NaHCO_3$ and brine, and dried over Na_2SO_4. The foamy residue was further purified by flash chromatography (Still et al., 1978) on a silica gel column with diethyl ether as mobile phase. The product was obtained as a foam after drying (Na_2SO_4) and evaporating the ether. Yield: 10.92 g (69.3%); R_f(A): 0.77.

L-Leucyl-L-alanine benzyl ester trifluoroacetate (TFA Leu-Ala-OBzl; 4) Compound **3** (10.80 g, 27.50 mmol) was dissolved in 35 ml of TFA and stirred at room temperature for 1 h, and the excess reagent was evaporated under reduced pressure. The residue was triturated with dry ether and the solid product dried overnight in vacuo over P_2O_5 and NaOH pellets. Yield: 10.62 g (94.5%); R_f(A): 0.15.

N-(tert-Butyloxycarbonyl)-L-alanyl-L-leucyl- L-alanine benzyl ester (Boc-Ala-Leu-Ala-OBzl; 5) The title compound was prepared from *N-tert*-butyloxycarbonyl-L-alanine (Boc-Ala) (1.86 g, 9.82 mmol), compound **4** (4.00 g, 9.82 mmol), DCC (2.33 g, 10.80 mmol), triethylamine (1.36 ml, 9.82 mmol) and HOBt (3.00 g, 19.64 mmol) as described for compound **3**. The product crystallized from ethyl acetate/petroleum ether. Yield: 2.11 g (46.3%); R_f(A): 0.65.

N-(tert-Butyloxycarbonyl)-L-alanyl-L- leucyl-L-alanine (Boc-Ala-Leu-Ala; 6) Compound **5** (5.00 g, 10.79 mmol) was dissolved in 50 ml of methanol and hydrogenated over 0.50 g of palladium charcoal for 3 h. The catalyst was filtered off, and the solvent evaporated. The residue was dissolved in 50 ml ethyl acetate, washed with 1 M citrate, dried over Na_2SO_4 and evaporated under reduced pressure. The resulting white foam solidified after drying in vacuum overnight. Yield: 4.34 g (107%); R_f(A): 0.40.

N-(tert-Butyloxycarbonyl)-L-alanyl-L- leucyl-L-alanyl-L- leucyl primaquine (Boc-Ala-Leu-Ala-Leu-PQ; 7) Leu-PQ (0.749 g, 2.01 mmol) was dissolved in 3 ml dry DMF at 0°C. Boc-Ala-Leu-Ala (0.750 g, 2.01 mmol) in 2 ml dry DMF and HOBt (0.615 g, 4.02 mmol) in 2 ml dry DMF were added. DCC (0.456 g, 2.21 mmol) in 2 ml dry

DMF was slowly added. The mixture was stirred under nitrogen for 3 h at 0°C and overnight at room temperature. The urea was filtered off, and the solvent evaporated under reduced pressure. The oily residue was dissolved in 50 ml ethyl acetate, washed with 1 M $NaHCO_3$, brine, 1 M citric acid and brine, and dried over Na_2SO_4. The product was obtained as a yellow-brown foam after evaporating the solvent. Yield: 0.434 g (29.7%); R_f(A): 0.56.

L-Alanyl-L-leucyl-L-alanyl- L-leucylprimaquine (Ala-Leu-Ala-Leu-PQ; 8) Compound **7** (0.909 g, 1.25 mmol) was dissolved in 10 ml TFA and stirred for 1 h at room temperature. The excess reagent was removed by evaporation under reduced pressure. The resulting oil was mixed with 50 ml 1 M $NaHCO_3$ and the product extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and evaporated, giving the product as a yellow-brown oil. Yield: 0.547 g (69.6%); R_f(B): 0.54. Analysis of the product by HPLC gave one major peak ($t_R = 16.93$ min). Amino acid analysis for Leu and Ala after coupling of the compound to microparticles gave: Ala, 49.49%; Leu, 50.50%.

Preparation of polyacryl starch microparticles

The microparticles were prepared by polymerization of acryloylated starch in an emulsion, as previously described (Laakso et al. 1986). Briefly, a solution of acryloylated starch in 5 ml phosphate buffer (pH 7.5) was homogenized in 300 ml of toluene:chloroform (4:1). Ammonium peroxidisulphate (final concentration 0.08 M) and TEMED were used to initiate the polymerization. This procedure typically yields microparticles of which about 80% have a diameter between 0.5 and 2.5 μm (Laakso et al., 1986). The microparticle composition is characterized by the D-T-C nomenclature and the amount of TEMED added in the preparation (D represents acryloylated starch (g/100 ml); T is the total concentration of acrylic groups expressed as acrylamide equivalents (g/100 ml); and C is the relative amount of crosslinking agent (% w/w)). The microparticles used in this study had a D-T-C value of 10-0.5-0, and 100 μl of TEMED was added. ^{14}C-labelled PSM were obtained by including acryloylated [^{14}C]starch in the monomer solution.

Coupling of ligands to microparticles

Ala-Leu-Ala-Leu-PQ was coupled to the microparticles essentially as described previously (Laakso et al., 1987). Microparticles (5 mg/ml) were activated with CDI (50 mg/ml) in dry DMF for 1 h at room temperature. After washings with DMF to remove unreacted CDI, 150 mg of the particles were suspended in 43 ml of DMF with 1 M TEMED containing 314 mg of the ligand, corresponding to 0.60 mol peptide per mol glucose, and rotated end-over-end overnight at room temperature. To block remaining active groups on the starch, the particles were washed with DMF, suspended in a 0.5 M sodium carbonate buffer (pH 10) containing 0.2 M glycine and 0.5% (w/w) Tween 20, and rotated end-over-end overnight at room temperature. The particles were then washed to physiological saline and stored at 4–6°C until use. Analyses of the amount of coupled Ala-Leu-Ala-Leu-PQ were made by amino acid analysis, and corresponded to a PQ diphosphate content of 9.74 μg/mg of microparticles. The enzymatic release of PQ from the microparticles was investigated by incubation in a lysosome-enriched fraction as described previously (Laakso et al., 1987). After 24 h incubation, 12% of the PQ content was released as free PQ as determined by HPLC.

Control microparticles, containing glycine only, were prepared by CDI activation of the microparticles as described above, and then suspending the particles in a 1 M sodium carbonate buffer (pH 10) containing 0.2 M glycine and rotated end-over-end overnight at room temperature.

Coupling of Ala-Leu-Ala-Leu-PQ to ^{14}C-labelled PSM was made as above. The PQ diphosphate content was 16.3 μg/mg and the specific radioactivity was 122000 dpm/mg.

Parasites

L. donovani MHOM/ET/67/HU3 (LV9) donated by Dr R.A. Neal, London School of Hygiene and Tropical Medicine, U.K., were maintained as amastigotes by serial passage in Syrian hamsters *(Mesocricetus auratus)*. A suspension of *L. donovani* (1 ml, 2.5×10^8 parasites) was injected intraperitoneally and the hamsters were killed about 8 weeks after infection. Amastigotes

were obtained by homogenizing the infected spleen in a Dounce homogenizer and removing tissue debris by centrifugating the suspension at $150 \times g$ for 40 min. The parasites were then washed twice in PBS by centrifugation at $1600 \times g$ for 20 min and resuspension. Viable amastigotes were counted under a fluorescence microscope after staining with fluorescein diacetate and ethidium bromide (Cenini et al., 1989).

Treatment of mice

Mice of the BALB/c A Bom strain (Bomholtgård, Ry, Denmark), 8–10 weeks old, were injected in the tail vein with 0.2 ml parasite suspension containing 1×10^7 viable amastigotes. The infected mice were injected in the tail vein with PQ-loaded PSM, empty PSM (containing glycine only), or free PQ in a volume of 0.20 ml on day 14, 16 and 18 after infection. Daily doses of both types of microparticles were 0.05, 0.2 and 0.5 mg of particles. Daily doses of free PQ diphosphate were 0.05, 0.2 and 0.5 mg. Groups of treated mice were killed and imprints taken 1 week after the final treatment (day 25 after infection). Untreated control groups were killed at day 14 (control 1) and day 25 (control 2) after infection. The visceral infection was determined by examining Giemsa-stained imprints of the cut liver. Liver parasite burdens were expressed as Leishman-Donovan units (LDU) using the formula: number of amastigotes per 1000 liver cell nuclei × organ weight (g) (Bradley and Kirkley, 1977). The effect of different treatments was expressed as per cent killing and calculated as:

$$[1 - (b/a)] \times 100$$

where a = LDU in control 2 and b = LDU in treatment group.

Distribution and elimination of microparticles in vivo

BALB/c mice (see above) were given 0.5 mg of radiolabelled PSM in 0.2 ml of physiological saline in the tail vein. Blood samples (150 μl) were taken from the retroorbital plexus and the mice were then killed by decapitation. The total radioactivity in the blood was calculated assuming a total blood volume of 7% of the body weight (Sluiter et al., 1984). The spleen, lungs, one kidney, and 0.15–0.20 g of the liver were removed and prepared for liquid scintillation counting as described earlier (Artursson et al., 1983). No correction was made for the radioactivity contribution from the blood in the organs.

Results

Organ distribution of PQ-PSM

The initial organ distribution of PQ-PSM was studied in infected and uninfected mice. The results are shown in Fig. 2. The typical RES distribution, as shown earlier with 'empty' PSM

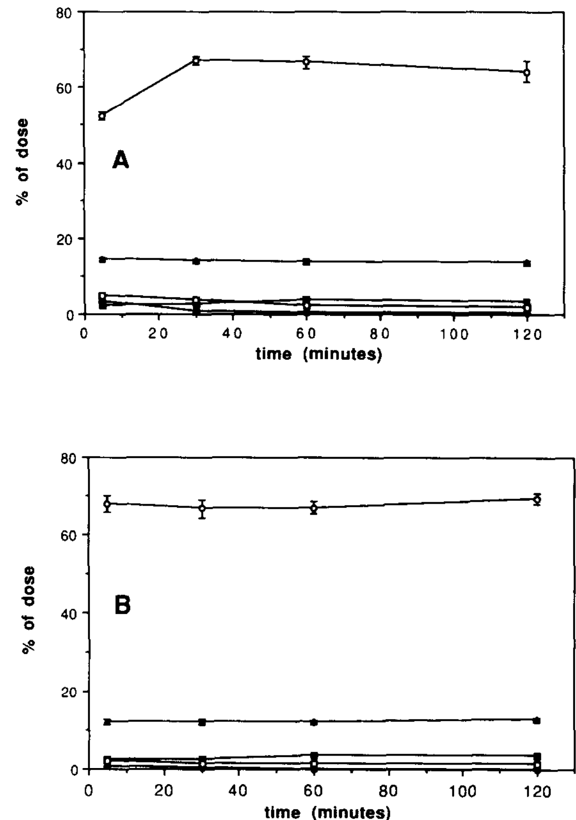

Fig. 2. Initial organ distribution of radioactivity after intravenous injection of ^{14}C-labelled PQ microparticles in uninfected (A) and infected (B) mice. The radioactivity in each organ was determined at the indicated times after injection of 0.5 mg microparticles as described in Materials and Methods. Each value is the mean ± S.E. of 6 animals. The organs were: blood (●); liver (○); spleen (■); kidneys (□); lungs (▲).

(Laakso et al., 1986) is maintained, with the exception of a higher lung uptake of PQ-PSM. The amount of radioactivity in the lungs had decreased to about 6% of the injected dose after 1 week. Also, the liver uptake in uninfected mice is somewhat slower compared to earlier results with empty PSM, where the maximal uptake was 5 min after injection.

Microparticles with coupled PQ have a tendency to aggregate, which can be avoided by including *N*-acryloylalanine in the microparticle preparation (Laakso and Sjöholm, 1987; Stjärnkvist et al., 1987). However, this will increase the content of synthetic hydrocarbon chains in the particles which probably make them less biodegradable. In this paper an alternative approach was introduced.

Apparently, coupling of PQ to the microparticles increased their hydrophobicity and consequently their tendency to aggregate, as could be observed by light microscopy. This was avoided by the inclusion of glycine as a blocking agent in the last step of the coupling procedure. After coupling of the PQ derivative, glycine is added and will react with the remaining active groups, thus increasing the hydrophilicity of the particles by its free carboxylic group. With this treatment, no aggregates was observed by light microscopy or Coulter-counter measurements. However, it cannot be excluded that a certain tendency to aggregate remained, which could explain the initial relatively high lung uptake. Additionally, it is known that PQ accumulates in the lungs after intravenous injection, and about 5% of the dose is found in the lungs between 20 and 120 min after administration (Pirson et al., 1982). Possibly, this property of free PQ could also influence the organ distribution of PQ-PSM.

As also seen in Fig. 2, the infection had no influence on the distribution of microparticles, at least not after 30 min. However, the 5 min values show that the liver uptake in infected animals is somewhat faster.

Elimination of PQ-PSM from the liver and the lungs

The fate of i.v. injected PQ-PSM in the liver and lungs was followed for 20 weeks (Fig. 3).

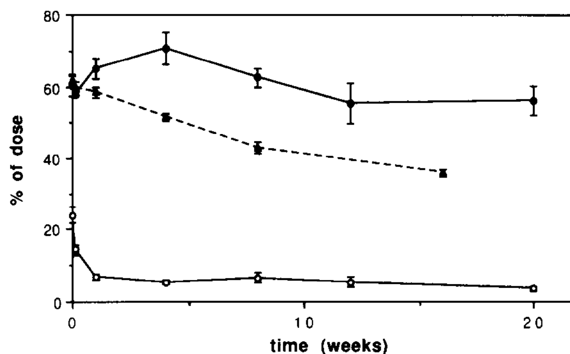

Fig. 3. Elimination of radioactivity from the liver (●) and the lungs (○) after intravenous injection of ^{14}C-labelled PQ microparticles in uninfected mice. The radioactivity in each organ was determined at the indicated times after injection of 0.5 mg microparticles as described in Materials and Methods. For comparison, the liver elimination of corresponding microparticles without PQ and glycine (microparticle I in Laakso et al., 1986) is indicated (△). Each value is the mean ± S.E. of 4–6 animals.

Compared to earlier results (Laakso et al., 1986) with microparticles prepared in the same way but without anything coupled, there are differences in the liver elimination. Firstly, there is an initial rise in the radioactivity which is not seen with microparticles without PQ. This could probably be due to a redistribution of the particles. A similar rise has been seen before with polyacrylamide microparticles (Sjöholm and Edman, 1979). Secondly, the elimination phase is slower for PQ-containing microparticles than microparticles without PQ.

The high initial radioactivity in the lung is only partly rapidly eliminated, and after 1 week, with about 7% remaining, a very slow elimination can be seen. In previous studies with 'empty' microparticles, the lung value was always below 1% after 24 h (Laakso, T., personal communication).

Treatment of L. donovani-infected mice

The antileishmanial effect of PQ-PSM, PSM with glycine only, and free PQ was determined in a mouse model. In preliminary experiments, the course of infection was studied in untreated mice (results not shown). The subline of BALB/c (BALB/c A Bom) used in our experiments was susceptible to *L. donovani* and during the first 4 weeks after infection a rapid visceral proliferation

of parasites was evident. However, after this period the parasite burdens declined and the mice entered a chronic or recovery phase, and between 6 and 12 weeks the infection was at a fairly constant level at about 15% of the maximum 4 week level. Thus, the mice have the cure phenotype, as described by Murray et al. (1982), as opposed to the non-cure BALB/c type, where a high infection level is retained up to 130 days after infection (Blackwell et al., 1980). Therefore, the treatment was started at day 14 after infection and the mice were killed at day 25 after infection, a time period where the infection has reached a manifest stage but not started to decline. In a typical infection the LDU in control mice was increased by a factor of 2–3 between days 14 and 25 (controls 1 and 2). LDU in controls at day 14 was in the range 1000–3000, and at day 25, 5000–10 000.

Three doses of microparticles and free PQ were administered. In preliminary experiments (results not shown), the maximum i.v. dose of free PQ without signs of acute toxicity in mice weighing about 20 g was found to be in the range 0.5–1.0 mg. According to Pirson et al. (1980), the highest i.v. dose without acute toxicity in mice is 25 mg/kg. Therefore, the highest daily dose of free PQ diphosphate in this study was 0.5 mg.

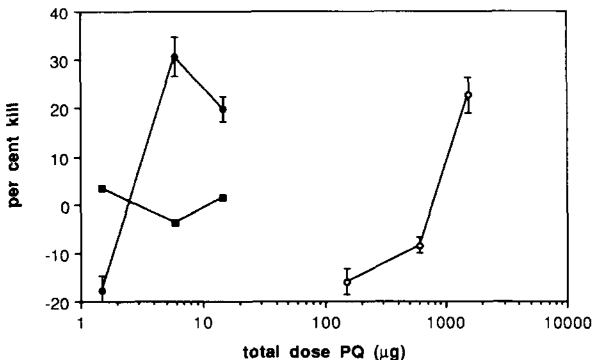

Fig. 4. Effect of free PQ (○), PQ microparticles (●) and empty microparticles in corresponding doses (■) on *L. donovani* in mice. Mice were infected on day 1 and treated on days 14, 16 and 18. The figure indicates the total dose. Number of parasites in the liver was determined at day 25 and per cent killing was calculated as described in Materials and Methods. Each value is the mean ± S.E. of 6 animals.

Likewise, the highest daily dose of microparticles was 0.5 mg, since this is the highest well tolerated dose studied (Laakso et al., 1988). The toxicity of PQ-PSM has not been investigated, but no sign of acute toxicity at the 0.5 mg dose was seen in this study.

The effect of different treatments is shown in Fig. 4. The efficiency of PQ to kill *L. donovani* in this model was greatly increased by coupling to PSM. No clear dose-response relationship was established, but the increase can be estimated to be about 200-fold. However, only a 30% reduction of parasite load was seen in this experiment, irrespective of whether or not PQ was bound to PSM. Particles carrying glycine only had no effect.

Discussion

In a previous study the antileishmanial effect of PQ-PSM was demonstrated in vitro, using mouse peritoneal macrophages (Stjärkvist et al., 1987). In view of these findings, we wished to investigate the efficiency of PQ-PSM in vivo, where the targeting effect of the carrier can be utilized. Also, it has been shown earlier that PSM can be used as a carrier for the antileishmanial drug sodium stibogluconate in vivo, increasing the efficiency of the drug by a factor of 100 (Baillie et al., 1987). PQ incorporated in liposomes has previously been used in the treatment of experimental murine malaria (Pirson et al., 1980). The incorporation did not increase the drug activity as compared with free PQ. However, the acute toxicity of PQ was reduced by a factor of 3.5 and subsequently higher doses were possible, increasing the therapeutic index. The lack of increase in activity was explained by a relatively low distribution of liposomes to the parenchymal cells, known to contain the sporozoites. In contrast, when liposome-encapsulated PQ was used to treat visceral leishmaniasis in hamsters (Alving et al., 1980), a greater efficacy was seen with the encapsulated drug. The liposome form was unable to increase the maximal parasite suppression by PQ (about 60%), but did enable a dose reduction by a factor of 100–1000.

In the present study, the targeting capacity of PSM was confirmed. A dose reduction by a factor of 200 was possible when PQ was coupled to PSM. Also, the ability of the peptide spacer coupling method to enable release of active drug inside lysosomes, earlier demonstrated in vitro (Stjärnkvist et al., 1987), was now shown in vivo. However, as also observed by Alving et al. (1980), 100% killing of the parasites was not achieved using PQ, and the carrier did not increase the maximal killing. In our system, this figure was about the same for free and microparticle bound PQ and varied between 30 and 70% in different experiments. This variation is supposedly due to the variation in infection virulence, as suggested by Alving et al. (1984). When the control LDU was low, a higher degree of killing was reached, and vice versa.

As seen before (Baillie et al., 1987), empty microparticles had no antiparasitic effect in vivo. Thus, the increased efficacy of microparticle-bound PQ is apparently due to a targeting to the Kupffer cells and a subsequent release of active drug in the lysosomes. This is supported by the high liver distribution of PSM shown by Laakso et al. (1986) and the selective uptake of PSM by Kupffer cells (Laakso and Smedsrød, 1987). As opposed to the findings in this study and that of Baillie et al. (1987), an earlier study using cultured peritoneal macrophages showed an anti-leishmanial effect of empty PSM (Stjärnkvist et al., 1987). This was explained by the release of hydrogen peroxide from the cells, induced by the interaction with the microparticles. However, the murine Kupffer cell, of interest in the present study, is impaired in its oxidative response to soluble and particulate stimuli (Lepay et al., 1985).

The interaction between intravenously injected colloids and the RES is affected by the surface characteristics, for example, charge and hydrophilicity, of microparticles (Wilkins and Myers, 1966) and liposomes (Gabizon and Papahadjopoulos, 1988). Regarding PSM, these characteristics can obviously be changed by the coupling of PQ and glycine. Therefore, it was important to investigate the rate of blood clearance and organ distribution of PQ-PSM, as has previously been done for empty PSM. However, these properties were not substantially changed as compared with the data of Laakso et al. (1986), except for a higher lung uptake, possibly owing to the increase in hydrophobicity due to PQ. Also, the infection itself did not greatly influence the organ distribution and rate of blood clearance of PQ-PSM. A somewhat faster liver uptake was seen in the infected animals, possibly due to stimulation of phagocytosis by the infection (Biozzi et al., 1957).

To determine whether the coupling of PQ influences the elimination of the particles from the body, the radioactivity in the liver and the lung was followed after injection of [^{14}C]starch PQ-PSM. Comparing earlier results with the same type of microparticles without PQ, the elimination of PQ-PSM was slower, from both the liver and the lungs. Presumably, the tetrapeptide-PQ derivative must first be cleaved from the particles before breakdown of the particle itself can take place. If the cleavage is a slow process, which the in vitro data indicate (only 10% of the PQ is released after 24 h), this could explain the slower elimination of PQ-PSM.

In conclusion, this paper confirms the ability of PSM to carry low molecular weight drugs to the liver macrophages and deliver them intralysosomally in an active form, thereby reducing the dose needed to achieve the desired effect of the drug. Thus, the PSM are shown to be an alternative to liposomes as a lysosomotropic drug carrier in intracellular parasitic diseases.

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