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Research paper

Characteristics of a novel phospholipid-based depot injectable technology for poorly water-soluble drugs

Harry Tiemessen, Peter van Hoogevest, Mathew L.S. Leigh*

Phares Drug Delivery AG, Muttenz, Switzerland

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Abstract

Phospholipid concentrates in a water miscible solvent were explored as injectable formulations for the poorly water-soluble drugs, using the anti-infective PHA 244 as model substance. Formulations containing up to 70% w/v phospholipid could dissolve 15% PHA 244. The formulations showed excellent syringe-ability and no precipitation of the drug after dilution in an excess of water. The local tolerability and pharmacokinetics of the formulations were explored after subcutaneous injection into cattle. A slow release pattern over a 2-week period and excellent local tolerability at the injection site were observed. Considering the low manufacturing costs, related to the production of solutions, this SupraVail™ MLM (Membrane Lipid Matrix) technology is a cost-effective alternative to more expensive depot technologies for poorly water-soluble drugs with similar release characteristics, like sterile aqueous and oily drug substance suspensions, as cited in the literature.

Keywords: Lipophilic drug; Phospholipid; Subcutaneous; Depot; Injectable; Local tolerability; Pharmacokinetics

1. Introduction

Optimal subcutaneous/intramuscular administration of poorly water-soluble drugs poses an enormous challenge in pharmaceutical and veterinary sciences. Since water cannot be used as a solubilisation vehicle, three general approaches to achieve subcutaneous administration can be explored. The first approach is to make an injectable solution by increasing the solubility of the drug in water through addition of co-solvents, cyclodextrins, detergents and mixed micelles [1–3]. The second approach is to use aqueous-based dispersed systems, such as oil-in-water emulsions [4], drug substance- [1] suspensions, liposomal- [5] and microparticle suspensions [6]. A further approach is to use non-aqueous vehicles or injectable suspensions like oil solutions, oil–drug substance suspensions [1] and in-situ formed implants [7]. Aqueous dispersed formulations, non-aqueous solutions and dispersions are mostly used as slow release formulations.

Oil solutions and drug substance suspensions control the release for weeks [1] whilst polymer-based microparticles and in-situ formed implants are claimed to last for months [7].

From a formulation perspective, solutions are easier to develop than the more complex suspension type of formulations. Unfortunately, to achieve adequate therapeutic drug levels of poorly soluble compounds, the solvent and/or detergent required may cause tissue irritation and tolerability problems at the injection site [8]. Furthermore, drugs with limited aqueous solubility may precipitate uncontrollably at the site of injection and possibly result in variable dissolution, release and bioavailability.

The objective of this study was to explore the characteristics of novel, solution type, phospholipid-based subcutaneous/intramuscular injection formulations for drugs with low water solubility with respect to local tolerability at the injection site and release characteristics. SupraVail™ MLM is a membrane lipid technology developed by Phares that maximises the bioavailability of poorly water-soluble drugs [9]. The technology is based on the preparation of drug–phospholipid complexes in which the drug is dissolved in the lipid (i.e. fatty acid) domain of the phospholipid membrane. These complexes

* Corresponding author. Phares Drug Delivery AG, P.O. Box, Kluenenfeldstrasse 30, CH-4132 Muttenz, Switzerland. Tel.: +41-61-317-9040; fax: +41-61-317-9050.
E-mail address: info@phares.biz (M.L.S. Leigh).

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hydrate in-vivo and convert into drug–lipid complexes which have been shown to have superior properties for more efficient membrane transfer [9].

The poorly water-soluble research compound PHA 244 which has anthelmintic activities and mainly intended for veterinary use in livestock, was used as model substance. The physicochemical properties of the phospholipid-based formulations and the in vivo performance of these formulations in cattle with respect to pharmacokinetics and local tolerability of the PHA 244 formulations were assessed in the current study.

2. Materials and methods

2.1. Materials

PHA 244 was synthesised using proprietary methods and had a purity of 99.5%. PHA 244 is an anthelmintic with MW of 360, a log *P* (octanol/water) of 6.3, and solubility in water at pH 7 and 20 °C of 0.1 ppm. Soy phospholipids (SPC; 96.6% phosphatidylcholine with 1.7% lysophosphatidylcholine) were purchased from Lipoid GmbH, Ludwigshafen, Germany. All other chemicals, solvents and excipients used were at least of analytical grade.

2.2. Methods of manufacture of test injectables for animal experiments

PHA 244 was mixed by stirring with a pre-determined volume of a solution containing varying amounts of phospholipids. All vials, stoppers, filters and other materials were sterilised by autoclaving. After heating to 60 °C, the clear solution was sterile filtered under laminar flow conditions through a 0.45 μm size filter into glass vials. Vials were purged with nitrogen and closed with rubber stoppers.

2.3. Analytical procedures

Solubility testing was performed by sequential addition of portions of liquid phase to a pre-determined amount of PHA 244. After each addition, the mixture was stirred for 10 min and examined visually for complete dissolution. Samples of clear formulations were diluted 2- or 10-fold with water. The resulting liposomal suspension was inspected microscopically for presence of crystals at regular time intervals.

Syringe-ability of the formulations was tested by using a 2 ml syringe equipped with a 40 mm, 18 G needle unless described otherwise. The needle was dipped into the test sample, which was kept upside down. Two millilitres vacuum was drawn and the time needed to fill the syringe with 1 or 2 ml of the test formulation was recorded. Results are presented as the time, in seconds, to withdraw 1 or 2 ml.

2.4. Animal experiments

Animal experiments were performed under the relevant guidelines of the European Community. The experimental protocols used were approved by the responsible ethical committee and complied with their recommendations.

2.5. Local tolerability testing

Ruminating male calves (Simmental/Red Holstein crossbred) were used for the study. The animals were approximately 5 months of age and weighed around 180 kg on treatment day. Only clinically healthy animals were selected for the experiment. One calf was used per treatment group. A corresponding volume of the 10% w/v PHA 244 formulations to give 10 mg drug/kg (2.5 ml per 25 kg body weight, i.e. 20 ml for 200 kg cattle) was administered subcutaneously into the left side of the calf, in an area just behind the shoulder. The same volume of the corresponding placebo formulations was injected into the same area on the opposite side of the same cattle. The general condition of the cattle, size of the swelling and formation of nodules and sensitivity of the site of injection to touch were recorded over a period of 11 days after injection.

2.6. Pharmacokinetic testing

Cattle were selected as for the local tolerability testing. Local tolerability testing and pharmacokinetic testing was performed in the same study with the same cattle. The cattle were allocated randomly into groups of 3 animals per treatment group. Test items were administered subcutaneously through a 14 G needle once using a dose of 12 mg drug substance/kg body weight. Since the test formulations contained 10% w/v PHA 244 a volume of 21 ml had to be administered for cattle weighing 180 kg. The animals were examined by a veterinarian prior to administration of test formulations as well as at set intervals to evaluate any changes at the injection site. Blood specimens of ca. 4.5 ml were also taken from the *V. jugularis* before treatment as well as 6, 12, 24, 28, 32 h and 2, 3, 4, 5, 7, 9, 14 and 21 days post-treatment. Plasma samples were analysed for PHA 244 and its active metabolite PHA 244 A using the following method: precipitation of proteins in 0.5 ml plasma by addition of 0.5 ml water (containing 0.2% trifluoroacetic acid) and 1.2 ml acetonitrile (containing 0.2% trifluoroacetic acid), centrifugation, extraction of the supernatant together with 5 ml water using C18 solid phase extraction cartridges (Varian Bondelut, 200 mg sorbent, Varian), (Middelburg, The Netherlands), washing with 2 ml acetonitrile/water 40:60 (v/v), elution with 3 ml acetonitrile (containing 0.2% trifluoroacetic acid), evaporation, dissolution of the residue in 125 μl of mobile phase, vortex mixing, then analysis by reversed phase HPLC-UV, using a Nucleosil C18 HD column (250 × 3 mm, Macherey-Nagel), (Oensingen,

Switzerland), with acetonitrile/40 mM diammoniumhydrogenphosphate (pH 7.75), 55:45 (v/v) as mobile phase and UV detection at 300 nm. Pharmacokinetic parameters were calculated individually for each animal using a standard software programme.

3. Results

Based on preliminary experiments, the efficacious dose of PHA 244 after oral administration of an aqueous suspension of micronised PHA 244 for treatment of infected cattle appeared to be ca. 10–12 mg drug substance/kg. In some cases, for convenience reasons and to guarantee efficacy, subcutaneous injection may be preferred to oral administration for veterinary products. In order to fulfil the requirement to have the smallest possible injection volume and simultaneously to achieve therapeutic levels, formulations containing ca. 10–20% PHA 244 should be explored.

Because of the high level of oral absorption and epithelial membrane transport, PHA 244 may have a strong interaction with phospholipid membranes. In order to assess the affinity of PHA 244 for phospholipids, ethanolic solutions containing 25 mg PHA 244/ml and phospholipids in weight ratios of 1:2, 1:5 and 1:10 and 1:20 were diluted 10-fold with an excess of water to form liposomes and observed for precipitation of the drug. Control experiments performed under the same conditions, but without phospholipids showed after dilution in all cases precipitation of the drug. It was found that the ratio of 1:10 or a higher excess of lipid versus PHA 244 gave rise to liposomal suspensions in which the drug was not precipitated but incorporated in the liposomes. Ethanolic solutions with a lower ratio of lipid to drug showed beside liposomes the presence of drug precipitates. Liposomes are, however, not suitable to formulate PHA 244 because the target drug concentration of at least 10% could not be achieved even when a typical lipid concentration of 10% w/v is used.

In contrast, SupraVail™ technology can employ up to 70% w/v phospholipids in water miscible solvents. Therefore, this technology may have a high solubilisation potential for PHA 244. PHA 244 is soluble in water miscible alkanols like propylene glycol (2.5%), methanol (5.5%), ethanol (7.5%) and isopropanol (12.5%). Ethanol was selected as water miscible solvent because of its acceptance by regulatory authorities in injectable formulations [8].

It was found that due to the presence of 50–70% phospholipids in the ethanol phase, the solubility of PHA 244 could be increased 2-fold to 15% w/v. Since the aim of the study was to develop an injectable formulation which after dilution in water does not show (or has a reduced risk for) an uncontrollable drug precipitation, an in-vitro assessment of the precipitation tendency of PHA 244 after dilution of the test formulations with water was an important optimisation criterion.

Formulations with phospholipid/ethanol/PHA 244 70/20/10 w/w (HT/00/10) appeared to be feasible with respect to drug concentration, viscosity, syringe-ability and delayed precipitation on dilution. A dilution of the formulation with one or 10 parts of water did not lead to precipitation of drug substance during 24 h at room temperature. Two further phospholipid-based formulations were made to study the extent of local tolerability in cattle. One contained additionally a detergent, polysorbate 80 (HT/00/09) to partially replace the phospholipid. The other contained oil (Miglyol™ 812) and ethyllactate (HT/00/11) to replace the ethanol. The formulation with oil and ethyllactate was clearly the most viscous formulation (see Table 1 for details). Also these two formulations did not show crystal formation for at least 24 h when diluted with water. None of the three formulations showed any precipitation of PHA 244 during storage at 4 °C or room temperature for 4 months.

The results of the local tolerability tests after subcutaneous injection (one cow per formulation) of the PHA 244 formulations (Table 1) at 10 mg/kg and corresponding placebos are listed in Table 2. The number of animals per groups was too low to allow statistical analysis of the results. The placebo formulations were very well tolerated. Only formulation HT/00/09 with PHA 244 showed unsatisfactory results compared to the placebo administered to the same cow. Eleven days after administration the presence of considerable sensitive swellings were observed. In agreement with previous experience, this effect was probably caused by precipitation of PHA 244 aggravated by the polysorbate 80. The formulation containing Miglyol and ethyllactate (HT/00/11) showed excellent tolerability but suffers from being too viscous. The formulation containing ethanol and phospholipid only (HT/00/10) showed acceptable local tolerability and acceptable viscosity for injection through a 40 mm, 14–18 G hypodermic needle.

Table 1
Characteristics of phospholipid-based formulations containing PHA 244 for local tolerability testing after subcutaneous administration in cattle

Formulation[a]	Composition		
	HT/00/09	HT/00/10	HT/00/11
PHA 244	10	10	10
SPC	50	70	54.5
Tween 80	20		
Miglyol 812			27.5
Ethanol (96%)	20	20	
Ethyllactate			8
Physical stability	Physical properties		
	>4 months 4 °C and RT	>4 months 4 °C and RT	>1 month 4 °C and RT
Syringe-ability 1 ml (s)	4	6	27
Syringe-ability 2 ml (s)	10	15	55

[a] Components in parts by weight.

Table 2
Local tolerability in cattle at the injection site after subcutaneous injection of phospholipid containing PHA 244 formulations at a volume of 18–22 ml and a dose of 10 mg PHA 244/kg

Cattle no.	150		153		161	
Formulations	Placebo HT/00/06	Active HT/00/09	Placebo HT/00/07	Active HT/00/10	Placebo HT/00/08	Active HT/00/11
Inj. side	Left	Right	Left	Right	Left	Right
Day 1	6.5 × 6.5[a] *[b]	7 × 7 *	8 × 8.5 *	9 × 8 *	7 × 7 *	7.5 × 7 *
Day 5	7 × 8 *	10 × 11 *	10 × 10 *	9 × 11 *	9 × 10 *	9 × 12 *
Day 7	7 × 8	10 × 11	8 × 8	7 × 6	8 × 6	5 × 6
Day 9	6 × 7	12 × 10 × 4 *	3 × 3	4 × 2	2 × 2	3 × 2
Day 10	5 × 4	10 × 7 × 3 *	2 × 3	3 × 2	2 × 2	3 × 2
Day 11	5 × 5	8 × 7 × 3	Nodule 2 × 4	Nodule 3 × 4	Very thin layer	Very thin layer

[a] Surface area of swelling in $l \times b$ in cm; In case significant swelling is observed the height (cm) of the swelling is mentioned.
[b] Slightly sensitive injection site.

Table 3 lists the characteristics of the selected formulations for further explorative testing of pharmacokinetic properties. Because of the negative results, detergents and ethyllactate were omitted from the selected formulations.

After parenteral and oral administration, PHA 244 is immediately metabolised to PHA 244A (which is active against parasites). This metabolism is also observed after oral administration of anthelmintics like albendazole [10], fenbendazole [11] and triclabendazole [12], which are structurally related to PHA 244. No original PHA 244 could be detected in plasma. In Fig. 1 the mean plasma profiles of the active metabolite PHA 244A in cattle of the subcutaneous formulations with PHA 244 are depicted. For comparison the pharmacokinetics of PHA 244 after oral administration of a 10% suspension of micronised PHA 244 (particle size 5–10 μm) in water are depicted as well. In Table 4 the corresponding pharmacokinetic parameters are listed.

From this figure and table the following can be derived. The half-life $t_{1/2}$ of the subcutaneous formulations was five to nine times longer compared to oral administration, suggesting that there is a pronounced slow release effect from the injection site. As a consequence, the profile of the active metabolite of PHA 244 reached a maximum (C_{max}) of only 1000–2300 ng/ml compared to 9000 ng/ml after oral administration. This slow release effect with lower C_{max} and higher T_{max}, was even more pronounced for formulation HT/00/42 compared to the other two formulations.

In general the lipophilic PHA 244 shows excellent bioavailability. The relative subcutaneous bioavailability versus oral bioavailability (AUC_{subcut}/AUC_{oral}) was approximately in the range of 0.77–0.88. This means that the bioavailability of the injectable formulation was almost as high as the oral formulation. Since the results are the mean of three animals per group, statistical analysis to determine the significance of these findings was not performed.

Table 3
Characteristics of phospholipid-based formulations containing PHA 244 for pharmacokinetic testing after s.c. administration in cattle

Formulation[a]	HT/00/42	HT/00/43	HT/00/44
PHA 244	10	10	10
SPC	70	60	55
Miglyol 810		15	20
Ethanol 96%	20	15	15
Physical stability	Physical Properties		
	>2 months 4 °C and RT	>2 months 4 °C and RT	>2 months 4 °C and RT
Syringe-ability 1 ml (s)	5	3	3
Syringe-ability 2 ml (s)	10	8	8

[a] Components in parts by weight.

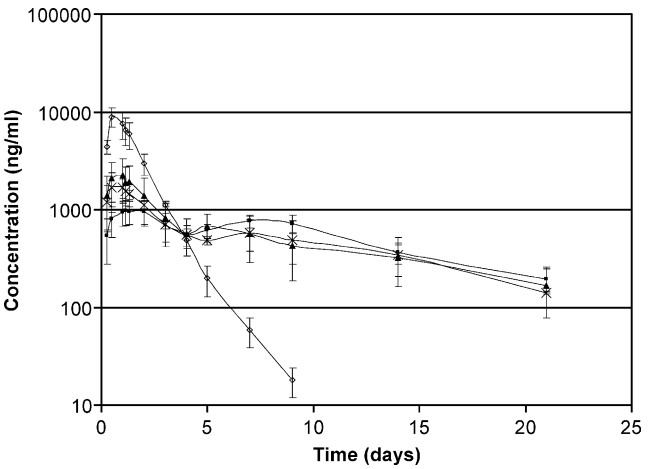

Fig. 1. Mean plasma profiles [ng/ml] ± SD ($n = 3$), of PHA 244A in cattle after subcutaneous administration of phospholipid-based injectable PHA 244 formulations (-■- HT/00/42; -▲- HT/00/43; - × - HT/00/44), compared to oral administration of a suspension of PHA 244 -◇.

Table 4
Pharmacokinetic parameters ± SD ($n = 3$) of PHA 244A after subcutaneous administration compared to oral administration of an aqueous suspension of PHA 244

Parameter	Suspension oral	HT/00/42 subcutaneous	HT/00/43 subcutaneous	HT/00/44 subcutaneous
T_{max} [d]	0.5 ± 0	3.4 ± 3.1	1.0 ± 0	0.8 ± 0
C_{max} [ng/ml]	8950 ± 2026	1044 ± 225	2266 ± 1088	1819 ± 586
AUC(0 − t) [d × ng/ml]	16199 ± 3529	11334 ± 1753	11998 ± 3250	10874 ± 1882
AUC(0 − inf) [d × ng/ml]	16223 ± 3526	13164 ± 2368	14225 ± 3063	12464 ± 1475
Relative bioavailability s.c./oral	1	0.81 ± 0.23	0.88 ± 0.27	0.77 ± 0.19
MRT [d]	1.4 ± 0.1	11.0 ± 1.1	11.1 ± 4.9	10.2 ± 0.5
$t_{1/2}$ [d]	0.9 ± 0.1	6.4 ± 0.6	8.8 ± 3.7	7.8 ± 2.1

4. Discussion

The feasibility of SupraVail™ MLM technology for oral administration has been demonstrated for the poorly water-soluble drug cyclosporine A [8]. In this present study it is also shown that SupraVail™ MLM injection technology may offer a viable alternative to the traditional approaches for formulating drugs with low water solubility for subcutaneous injections.

After subcutaneous administration of the model substance PHA 244 formulated in the phospholipid-based formulations in cattle, it appears that the selected formulations do not allow a fast onset of action nor a burst effect, but rather, a slow release pattern of the drug over a period of about 2 weeks. This slow release pattern can be explained by the formation of a viscous semi-solid lipid reservoir at the injection site from which the drug is slowly released into the tissue fluids. In this regard the SupraVail™ formulations may be an alternative to oily or aqueous suspensions for injections of lipophilic drug substances, which also have depot injectable properties [13–16].

Whether, the observed blood levels of PHA 244 are sufficient to be efficacious against infectious organisms is the subject of further study. In general, the formulations comprising phospholipids as the only solubiliser (Table 2, formulations HT/00/07, -/08, -/10, -/11) showed an excellent local tolerability at the injection site. Formulations which contained detergents beside phospholipids showed a much lower degree of local tolerability (Table 2, formulations HT/00/06 and -/09).

SupraVail™ MLM injection technology may be considered as an in-situ forming depot formulation in similar fashion to the biodegradable polymers and *N*-methylpyrrolidone solvent approach [7]. Because of the biodegradability of the lipid used and their liquid crystalline character the duration of the release is maximally about 2 weeks at physiological temperatures in the test subjects. In this regard the SupraVail™ formulations may be compared with oily or aqueous suspensions for injections of lipophilic drug substances, which show similar release periods.

Other formulation technologies which use phospholipids to retard the release of water-soluble drugs after s.c. or i.m. administration are vesicular phospholipid gels [17,18], multi-vesicular liposomes [19–32] and liposomal suspensions [33–35]. The slow release properties of these vesicular formulations are not described for poorly water-soluble compounds. Since the release mechanisms of water-soluble drugs (i.e. passage trough liposomal membranes) are different compared to poorly water-soluble drugs (i.e. transfer from a liposomal membrane to other lipid molecules [36]) a further comparison of the slow release properties of SupraVail™ technology with liposomes is difficult to make.

In comparison with oily or aqueous suspensions for injection, SupraVail™ formulations are solutions which are much easier to prepare and sterilise by means of sterile filtration. Expensive preparation of sterile drug substance crystals can be avoided. The high phospholipid concentrations employed allow a high load factor for drugs with good lipid affinity. With PHA 244 this could be as much as 15% w/v which is comparable with the usual drug concentrations in suspension type formulations. Given the high lipid concentration, the formulations still have a low viscosity and very good syringe-ability.

The excellent local tolerability observed at the injection site of the phospholipid formulations may be explained by the binding effects of phospholipids to PHA 244 which prevents precipitation and possibly crystallisation. This in-vitro behaviour correlates well with in-vivo performance. To prevent precipitation/crystallisation of the drug a sufficient excess of lipid should be present in the formulation. In some cases this may be too low or the detergents present may interfere with drug lipid aggregate formation which protects the drug. As a result local tolerability is decreased (Table 2).

The high relative bioavailability (AUC_{subcut}/AUC_{oral}) of the injections tested suggests that PHA 244 is released from the formulation to a large extent. Although the SupraVail™ formulation contains ethanol the local tolerability of the vehicle itself, in spite of the large injection volumes, is excellent as well. This might be explained by the high concentration of phospholipid in the formulation and the strong interaction between ethanol and phospholipids [37]. After injection of the ethanolic solution, the ethanol diffuses only slowly from the lipids to the surrounding cellular lipid membranes. In this way there is no sudden increase of local

ethanolic concentration and tissue irritancy minimised, and cell damage avoided.

The lipid to drug ratio required to prevent precipitation of the drug is about 5. Taking into account that the special phospholipids from natural sources used in SupraVail™ are relatively much less expensive than synthetic phospholipids, the cost of good for such depot formulations are affordable.

In conclusion, phospholipid concentrates in water miscible solvents are suitable subcutaneous injectable formulations for poorly water-soluble drugs with high lipid affinity like PHA 244. As demonstrated taking PHA 244 as a poorly water-soluble model substance, SupraVail™ formulations do not cause local tolerability problems at the injection site. The vehicle itself is well tolerated and precipitation of the drug substance is prevented. Formulations with high concentrations of drug can be prepared which are still syringeable. Upon dilution with water, drug–lipid aggregates are formed which prevent drug crystallisation and related local inflammatory reactions. Furthermore, the formulations show a slow release of the drug over a 2-week period. Considering the low cost of goods and uncomplicated manufacturing method, these types of formulation offer a viable alternative to suspensions for injection for poorly water-soluble drugs like steroids/hormones, neuroleptics, anti-infectives and anti-cancer compounds (e.g. 4-hydroxyandrostenedione) in the veterinarian as well as human area.

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