International Journal of Pharmaceutics 416 (2011) 471–477

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Optimizing the therapeutic index of liposomal glucocorticoids in experimental arthritis

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ARTICLE INFO

Article history:
Received 27 October 2010
Received in revised form 11 March 2011
Accepted 11 March 2011
Available online 2 April 2011

Keywords:
Liposomes
Glucocorticoids
Rheumatoid arthritis
Therapeutic index
Targetting

ABSTRACT

Small-sized (less than 150 nm) long-circulating liposomes (LCL) may be useful as drug-targeting vehicles for anti-inflammatory agents in arthritis, since they selectively home at inflamed joints after i.v. administration. Previously it was shown in experimental arthritis that encapsulation of glucocorticoids (GC) as water-soluble phosphate esters in PEG–liposomes resulted in a strong improvement of the anti-inflammatory effect as compared to the free drug. In the present study, we compared the therapeutic activity and adverse effects induced by 3 different GC encapsulated in LCL in an attempt to further optimize the therapeutic index of liposomal GC in arthritis. Our data showed that with GC (dexamethasone, budesonide) of higher potency than prednisolone, the therapeutic activity of liposomal GC can be increased. However, side effects at the level of body weight and hyperglycemia were noted, related to the sustained free GC level observed after injection of the liposomal GC. An inverse relationship with the clearance rate of the free GC in question was shown. This study stresses the importance of a high clearance rate of the GC to be encapsulated for achieving a maximal therapeutic index with liposomal GC. Therefore high-clearance GC, which until now are only applied in local treatment approaches, may be very useful for the development of novel, highly effective anti-inflammatory preparations for systemic treatment of inflammatory disorders.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disorder, involving joint inflammation and progressive cartilage destruction (Bodman and Roitt, 1994). Glucocorticoids (GC) are highly effective anti-inflammatory drugs but their use in arthritis therapy is controversial due to a high incidence of serious adverse effects occurring during chronic treatment (Kirwan and Russell, 1998; Laan et al., 1999; Saag, 2002). As a result of rapid elimination from the circulation and unfavorable tissue distribution, systemic treatment with GC results in poor target localization of the drug, which often necessitates the use of high doses and intensive dosing schedules (Narang et al., 1983; Weusten et al., 1993). Targeted delivery of GC can greatly increase the concentration of the drug in the inflamed joints and therefore a less intensive dosing regimen may be sufficient for an adequate therapeutic response with minimal risk for side effects (Schiffelers et al., 2006).

Long-circulating liposomes have been extensively studied as targeted drug carrier systems in oncology and infectious diseases. Small-sized PEGylated liposomes (less than 150 nm) have been shown to selectively accumulate at the corresponding sites of pathology (Dams et al., 1999; Gabizon et al., 1994; Laverman et al., 1999; Schiffelers et al., 1999). The phenomenon of selective targeting to pathological sites can be attributed to locally enhanced permeability of the vascular endothelium, allowing small-sized PEG–liposomes to extravasate and accumulate in the extravascular tissue (Allen, 1997; Boerman et al., 1998). Larger sized liposomes, on the other hand, have a reduced circulatory half life and accumulate to a higher extend particularly in the spleen, at levels that can trigger high-dose glucocorticoid effects like inhibition of arachidonic acid release and alternative macrophage polarization to the M2 type. They also result in a lower degree of localization in the inflamed joints (Metselaar et al., 2003; Rauchhaus et al., 2009a).

Previous studies have shown that a single i.v. injection of GC in small-sized long-circulating liposomes yields a rapid, complete

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doi:10.1016/j.ijpharm.2011.03.025

and durable disease remission in a rat model of adjuvant arthritis (AA). Prednisolone was selected as model GC and the water-soluble inactive phosphate ester prednisolone phosphate (PLP) was encapsulated to achieve a stable liposome formulation. In its free form, PLP is quickly converted into active prednisolone in blood or tissues. Intensive treatment with repeated daily injections of free PLP could by far not match the effect of a single dose of the liposomal drug. Pharmacokinetic analysis of blood samples taken after administration of liposome-encapsulated PLP revealed not only the presence of liposome-bound PLP but also a low, sustained level of unencapsulated prednisolone in the circulation. The low, sustained level of free prednisolone did not contribute to the therapeutic activity of liposomal PLP (Metselaar et al., 2003).

A drawback for the translation of therapeutic effects observed in rat models to humans is that the pharmacokinetic behavior of prednisolone in rats is strongly different from that in human beings (Nichols et al., 1989; Schimmer and Parker, 2001; Thummel and Shen, 2001). Therefore, in the present study, we selected dexamethasone phosphate (DXP) for incorporation in liposomes, as the pharmacokinetics of dexamethasone in rats are more comparable to its pharmacokinetics in humans (Puisset et al., 2004; Varma and Mulay, 1980).

First, we investigated in the AA model whether incorporation of DXP in small-sized poly(ethylene glycol) (PEG) liposomes is also therapeutically beneficial. In view of the stronger potency of dexamethasone over prednisolone higher therapeutic efficacy was anticipated. In previous studies liposomal DXP was shown to be five times more effective compared to liposomal PLP, but it was associated with increased systemic adverse effects, likely due to higher sustained free drug levels (Schiffelers et al., 2006).

Second, it was evaluated whether the sustained free drug level observed after i.v. administration of liposomal GC is related to the occurrence of systemic side effects. As parameters for systemic side effects, treatment-induced loss of body weight and increase of blood glucose concentration were measured as these parameters can be quickly, frequently and accurately measured (as shown by Kaur et al. (1989) and Ogawa et al. (1992), respectively).

It was investigated whether the use of a high clearance GC could improve the therapeutic index, by reduction of the occurrence of side effects. The sustained free drug level correlated with the clearance rate of the free GC that appears in the circulation after injection of the corresponding liposomal formulation. The clearance rate is an important determinant of the free drug level, and therefore the chance of systemic side effects may be minimized by encapsulation of a GC with a high-clearance rate. Budesonide sodium phosphate (BUP) was selected as GC with a high clearance rate in this study. BUP has already shown to be more effective than liposomal PLP and DXP in a liposomal formulation in a melanoma B16 murine tumor model, without the occurrence of more adverse effects (Banciu et al., 2007, 2008). In this study we compared in the AA model small-sized PEG–liposomes containing DXP (relatively slow clearance rate) to small-sized PEG–liposomes containing GC with a higher clearance rate but a lower potency (PLP), and a GC with a higher clearance rate and a higher potency (BUP), with respect to pharmacokinetics, therapeutic response, and systemic side effects, such as body weight loss and increased blood glucose values.

2. Materials and methods

2.1. Preparation of liposomal GC

Small/sized long-circulating PEG–liposomes were prepared by the film-extrusion method (Amselem et al., 1993). Briefly a lipid solution was prepared in ethanol, containing dipalmitoyl phosphatidylcholine (DPPC) (Lipoid GmbH, Ludwigshafen, Germany), cholesterol (Sigma Chemical Co., Poole, UK) and distearoyl phosphatidylethanolamine–PEG 2000 (PEG–DSPE) (Avanti Polar Lipids, Alabaster, AL, USA) in a molar ratio of 1.85:1.0:0.15, respectively. The lipid solution was transferred to a round-bottom flask and a lipid film was created by rotary evaporation. The film was hydrated with a solution of 100 mg/ml of prednisolone disodium phosphate (PLP), dexamethasone disodium phosphate (DXP) (both obtained from Bufa, Uitgeest, The Netherlands) or budesonide disodium phosphate (BUP) (synthesized by Syncom, Groningen, The Netherlands) dissolved in sterile water. The resulting lipid dispersion was sized to a diameter between 90 and 100 nm by multiple extrusions through polycarbonate filter membranes. The unencapsulated GC-phosphate was removed by dialysis against 0.9% phosphate buffered saline using Slide-A-Lyzer dialysis cassettes with a molecular weight cut-off of 10,000 (Pierce, Rockford, IL, USA). The mean particle size was determined by dynamic light scattering with a Malvern 4700 system (Malvern Ltd., Malvern, UK). The phospholipid content was determined with a phosphate assay (Rouser et al., 1970) in the organic phase after extraction of liposomal preparations with chloroform. The aqueous phase after extraction was used for determining the GC-phosphate content by high performance liquid chromatography, using an isocratic system with a mobile phase of acetonitril–water with a pH of 2, followed by UV-detection at 254 nm. The liposomal preparations contained between 3.5 and 4.5 mg GC-phosphate and an average of 60 μmol phospholipid/ml.

2.2. Rat adjuvant arthritis

Male inbred Lewis rats between 7 and 9 weeks of age (170–200 g) were obtained from Maastricht University, Maastricht, The Netherlands. To induce arthritis, 100 μl of incomplete Freund's adjuvant (IFA) containing 10 mg/ml of heat-inactivated Mycobacterium tuberculosis (Mt) (both purchased from DIFCO laboratories, Detroit, MI, USA) was injected intracutaneously at the base of the tail (Koga and Pearson, 1973). At day 10 after the immunization, the first signs of joint inflammation became visible, together with a loss of body weight as a result of the disease. 20 days post-immunization the disease reached maximal severity, after which the inflammation process gradually resolved. Starting at day 10, the rats were daily examined for the visual signs of inflammation and the disease-induced weight drop. The severity of the joint inflammation was graded by assigning a score to each paw from 0 to 4, based on erythema, swelling and deformation of the joints (Koga and Pearson, 1973). The sum of these four grades for each animal is the clinical score and can vary from zero up to 16. Besides the development of paw inflammation, the disease results in a loss of body weight that could easily be monitored by daily weighing of the rats. The Dutch Committee of Animal Experiments approved all animal studies.

2.3. Therapeutic activity

All rats were treated on day 14 or 15 post-immunization, when the average score of all rats in the experiment reached 7, which were approximately half the maximal scores reached in these experiments. At the day of treatment, groups of five rats were formed with equal average clinical scores. All preparations were given intravenously in the tail vein. As the pharmacokinetics of PEG–liposomes have been shown to be lipid dose-independent, the administered dose of phospholipid was allowed to vary with the different liposomal GC preparations (Allen and Hansen, 1991). When daily injections of free GC were given, each following day treatment was repeated at the same time. The effect of treatment on clinical scores and body weight loss was monitored daily from

day 10 until day 30 post-immunization. Control rats were treated with 150 μmol total lipid/kg empty PEG–liposomes.

2.4. Systemic adverse effects

As parameters for systemic activity loss of body weight and increase in blood glucose concentrations were evaluated. Loss of body weight is a phenomenon that is generally observed upon systemic GC treatment in rats (Kaur et al., 1989). In this study the loss of body weight because of treatment with GC was clearly additional to the weight loss resulting from the induction of experimental arthritis. Besides the induction of body weight loss, systemic GC treatment can induce hyperglycemia (Ogawa et al., 1992). Monitoring the increase of blood glucose was performed by using a blood glucose meter (EuroFlash, LifeScan Inc., Miltiplas, USA).

2.5. Determination of liposomal GC-phosphate and free GC in the circulation

Previously it was shown that PLP remains stably entrapped in PEG–liposomes upon i.v. injection, since at different time-points post-injection PLP was detected in the same quantities as a liposome bilayer marker (assuming that unencapsulated PLP is quickly and completely converted to prednisolone after entering the circulation). Complete retention in the liposomes may also be expected with both DXP and BUP in PEG–liposomes. Despite this, very low levels of free GC in the plasma were observed with liposomal PLP, though these levels were found to not significantly contribute to the increased therapeutic effect of liposomal PLP (Metselaar et al., 2003). However, although not evaluated so far, they may contribute to the induction of systemic adverse effects. To evaluate this, plasma concentration–time curves of the different liposomal GC-phosphates were measured after injection of a dose of 10 mg/kg in healthy rats and were compared to PLP–PEG–liposomes. Concentrations of liposomal GC-phosphates were determined by plasma extraction followed by HPLC-determination (Derendorf et al., 1986). Concentrations as low as 200 ng/ml could be measured accurately. Quantities of free GC after injection of 10 mg/kg liposomal GC-phosphate in healthy rats could simultaneously be detected with the same assay in a single run with the phosphate ester. Concentrations of free GC in the extracts could be determined accurately down to a concentration of 50 ng/ml.

2.6. Statistical analysis

For statistically assessing and comparing therapeutic efficacy in different groups the nonparametric Wilcoxon/Kruskal–Wallis test (rank sums) was used. For evaluating differences between groups regarding other parameters, a one-way analysis of variance or a Student's *t*-test was performed. *P*-values of less than 0.05 were considered significant.

3. Results

3.1. Therapeutic activity in rat AA: DXP–PEG–liposomes vs. free DXP

Fig. 1 shows the anti-inflammatory effect of 2 mg/kg dexamethasone phosphate (DXP) i.v. encapsulated in PEG–liposomes and in free form. A single dose of 2 mg/kg free DXP significantly suppressed paw inflammation during three days. The same dose encapsulated in PEG–liposomes resulted in complete disappearance of the clinical signs of AA within two days. Complete remission of the disease symptoms lasted until day 20 (6 days post-treatment) after which joint inflammation gradually reappeared, reaching the inflammation score of the saline control group around day 24. The same therapeutic response could be realized by 5 daily injections of 2 mg/kg free DXP.

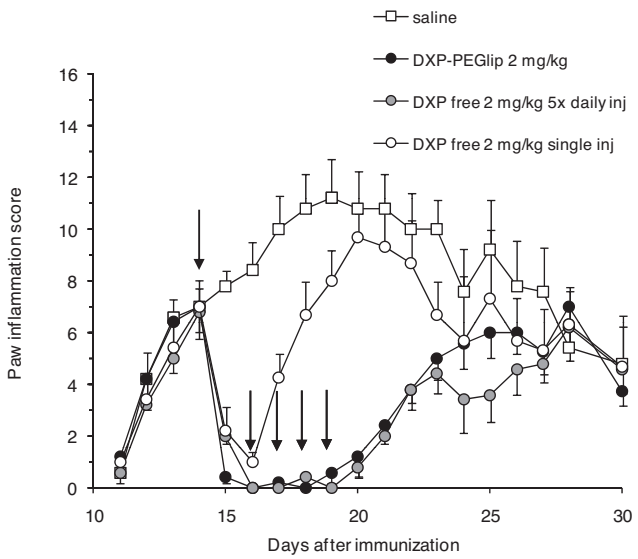

Fig. 1. Therapeutic activity in rat AA of 2 mg/kg DXP–PEG–liposomes vs. 2 mg/kg free DXP given as single or multiple daily treatment. Means of 5 rats are shown. Vertical bars show SEM. Arrow indicates first day of treatment.

3.2. Adverse effects: DXP–PEG–liposomes vs. free DXP

Fig. 2A shows the effect of the treatment on the total body weight of AA rats. Both liposomal DXP as well as multiple and single treatment with free DXP resulted in treatment-induced weight loss additional to the body weight loss as a result of the disease (saline treatment). Although the same effect was reached at the therapeutic level, repeated administration of 2 mg/kg free DXP generated a stronger treatment-induced loss of body weight than a single injection of 2 mg/kg liposomal DXP ($P < 0.05$ at day 18 post-immunization). In Fig. 2B it is shown that 2 mg/kg DXP in liposomal and in free form (single and repeated injections) enhanced blood glucose levels to a similar extent during the first days after treatment.

3.3. Therapeutic activity and adverse effects: PLP–PEG–liposomes vs. DXP–PEG–liposomes

To investigate the role of clearance rate of the free GC in the therapeutic index of liposomal GC, prednisolone phosphate (PLP) was encapsulated. However, as these two GC are known to differ in potency, first the therapeutic activities of liposomal PLP and liposomal DXP were compared. Fig. 3A shows the comparative effect of a single dose of 2 mg/kg and 10 mg/kg of PLP–PEG–liposomes and DXP–PEG–liposomes on rat adjuvant arthritis scores. There seems to be a correlation between the administered liposomal dose and the resulting therapeutic effect for both liposomal GCs. A dose of 10 mg/kg PLP–PEG–liposomes was equally effective as 2 mg/kg DXP–PEG–liposomes, indicating that liposomal PLP is approximately 5 times less potent than liposomal DXP.

Fig. 3B shows that 10 mg/kg PLP–PEG–liposomes reversed the disease-induced process of body weight loss between day 2 and 7 post-treatment. A dose of 2 mg/kg DXP–PEG–liposomes produced a similar response, however, body weight gain started 2 days later, between day 19 and day 24. In the period between day of treatment (day 15) and body weight gain, an additional loss of body weight was observed with liposomal DXP, which was not significant with liposomal PLP. Rats treated with 2 mg/kg liposomal DXP

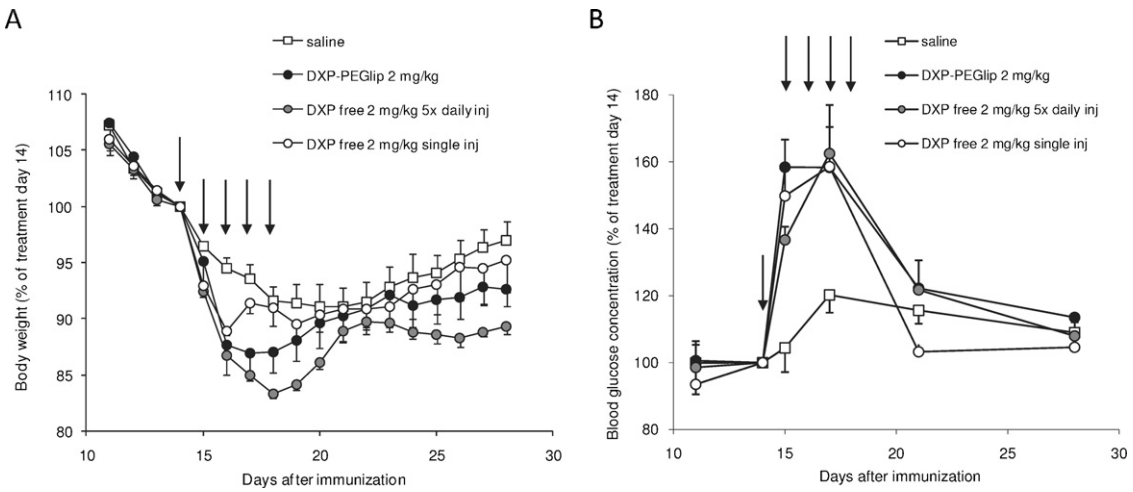

Fig. 2. Systemic effects as a result of 2 mg/kg unencapsulated DXP (single and multiple dose), and 2 mg/kg DXP–PEG–liposomes. (A) Effect on total body weight. Vertical bars show SEM. (B) GC-induced hyperglycemia. The percentage of the blood glucose concentration at day of treatment is shown. Vertical bars show SEM. In both A and B means of 5 rats are shown. Arrows indicate treatment days.

showed an additional weight loss of up to 5.5% as compared to rats in the control group over a period of 4 days before body weight gain was observed. With 10 mg/kg liposomal DXP, this additional body weight loss even reached 9.2%, lasting for more than a week.

3.4. Therapeutic activity and adverse effects: BUP–PEG–liposomes vs. DXP–PEG–liposomes

BUP is known to have a higher clearance rate, compared to DXP, and a higher potency, compared to PLP, and could therefore improve the efficacy and therapeutic index of the liposomal GC even further. The anti-inflammatory effect of a dose of 1 mg/kg BUP–PEG–liposomes was compared to the effect of 1 mg/kg and 2 mg/kg DXP–PEG–liposomes in the rat AA model (Fig. 4). Both liposomal DXP and liposomal BUP were highly effective in AA, causing a complete remission of joint inflammation. Liposomal BUP at a dose of 1 mg/kg is equally effective as liposomal PLP at a 10 fold higher dose (Figs. 3A and 4A). Importantly, Fig. 4B shows that 1 mg/kg liposomal BUP induced an almost complete regain of the disease-induced loss of body weight as a result of its therapeutic effect. This was also seen for liposomal PLP at a dose of 10 mg/kg (Fig. 3B). However, the opposite is observed after both 1 mg/kg and 2 mg/kg liposomal DXP, which induced an additional treatment-induced body weight loss. The reversal of disease-induced body weight loss as a result of the therapeutic effect was also seen with liposomal DXP, but this was occurring after the period of additional treatment-induced body weight loss.

3.5. Plasma concentrations: liposomal GC vs. free GC

Fig. 5A shows the plasma concentration–time profile of the three different GC-phosphates: PLP, DXP and BUP, after injection of a dose of 10 mg/kg encapsulated in PEG–liposomes. All three liposomal GC followed the same plasma concentration–time profile.

Despite equal dose and identical plasma concentration–time profile of the three liposomal GC-phosphates, strong differences were observed regarding the plasma concentration–time profile of the free (i.e., not bound to liposomes) parent drug detected in the circulation after treatment with the liposomal formulations (Fig. 5B). Treatment with liposomal DXP yielded the highest free drug levels, whereas treatment with liposomal BUP and PLP resulted in similar, but much lower levels of free GC. Roughly, the areas under the plasma concentration–time curves of free GC in the circulation were inversely correlated with the reported clearance

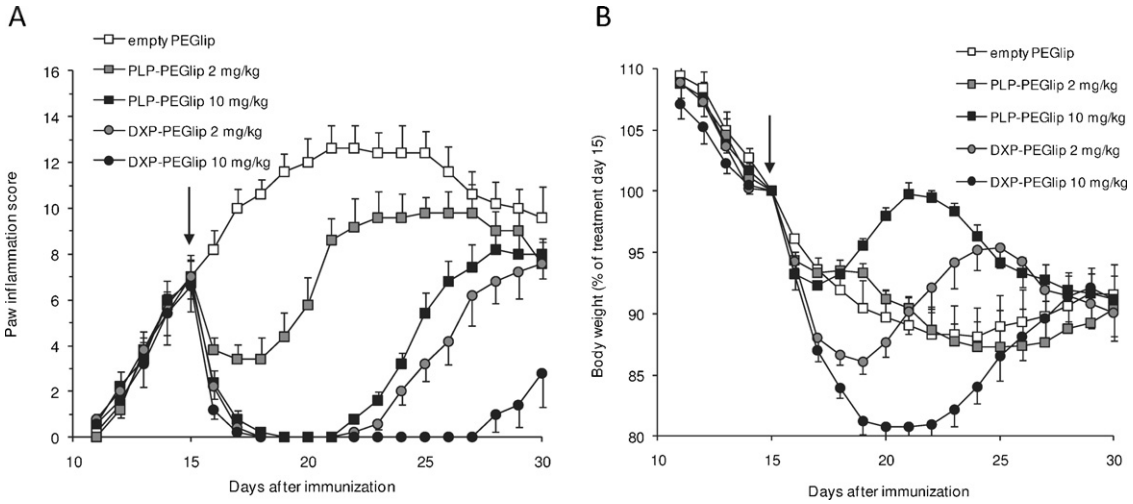

Fig. 3. Relative potency of PLP–PEG–liposomes vs. DXP–PEG–liposomes. (A) Effect on joint inflammation in rat adjuvant arthritis, and (B) effect on body weight. Body weight is shown as percentage of the body weight at day of treatment. Means of 5 rats are shown. Vertical bars show SEM. Arrow indicates treatment (day 15).

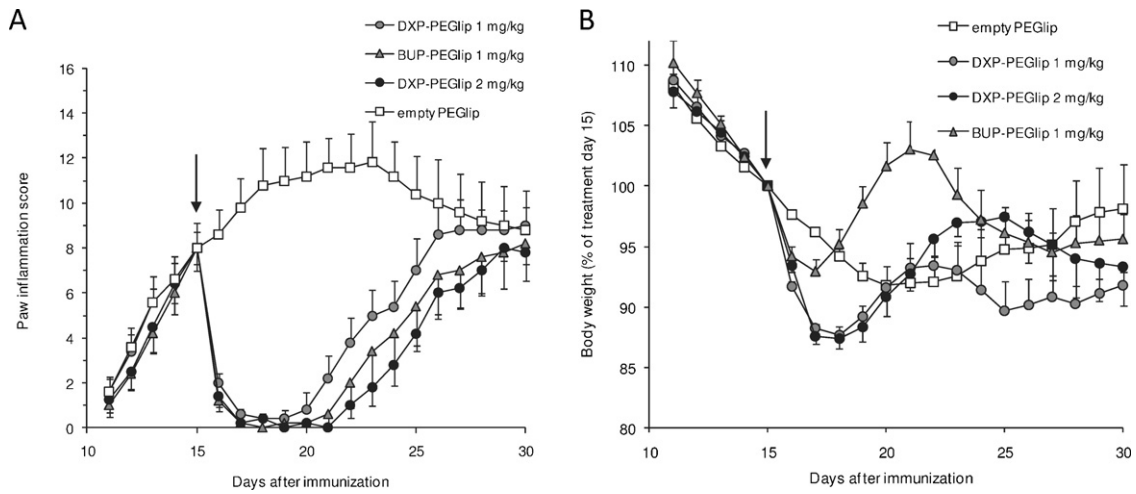

Fig. 4. Relative potency and systemic activity of BUP–PEG–liposomes vs. DXP–PEG–liposomes. (A) Effect on joint inflammation, and (B) effect on body weight of 1 mg/kg liposomal BUP and 1 and 22 mg/kg liposomal DXP. Body weight is shown as percentage of the body weight at day of treatment. Means of 5 rats are shown. Vertical bars show SEM. Arrow indicates treatment (day 15).

values (in rats: approx. 0.2 L h^{-1} kg^{-1} for dexamethasone (Varma and Mulay, 1980), 1.5 L h^{-1} kg^{-1} for budesonide (Chanoine et al., 1991) and 2.3 L h^{-1} kg^{-1} for prednisolone (Nichols et al., 1989)).

4. Discussion

In previous studies it was shown that small-sized long-circulating PEG–liposomes extravasate into inflamed joints in experimental rat and murine models of arthritis. Liposomal encapsulation of PLP enhanced the local anti-inflammatory activity to such an extent that a single i.v. injection of 10 mg/kg liposomal PLP was sufficient to induce complete, rapid and long-lasting remission of joint-inflammation. In the present study, we compared the therapeutic activity and adverse effects of 3 different GC, encapsulated in water-soluble phosphate form in small sized long-circulating liposomes in an attempt to further optimize the efficacy of liposomal GC in arthritis. However, increased efficacy is only clinically relevant when the adverse effects are not increased such that the therapeutic index of the drug is not improved upon liposomal encapsulation.

Dexamethasone was chosen as model GC, as its pharmacokinetics in rats are quite similar to that in humans. The first objective was to find the dose at which liposomal DXP induced a therapeutic response comparable to liposomal PLP at a dose of 10 mg/kg. It appears that a single i.v. injection of only 2 mg/kg DXP in small-sized PEG–liposomes can induce a full disease remission for almost a week, indicating that liposomal DXP is indeed approximately 5 times more potent than liposomal PLP (McKay and Cidlowski, 2000). Therapeutic benefit could also be realized with free DXP. However, 5 daily injections of 2 mg/kg were required to produce the same response as a single treatment with 2 mg/kg liposomal DXP, indicating that liposomal encapsulation strongly enhances the therapeutic activity of the drug (Fig. 1).

The second objective of this study was to evaluate possible systemic side effects induced by the sustained level of free GC in the circulation after injection of liposomal GC. First, the effect on body weight was evaluated. Besides paw inflammation, induction of AA in rats generally leads to a gradual fall of body weight. Therapeutic activity in the model is not only detectable by reversal of paw inflammation, but also by reversal of disease-induced body weight fall (Koga and Pearson, 1973). Reversal of body weight fall was clearly observed in a previous study with PLP–PEG–liposomes. In the present study, however, instead of a reversal, liposomal DXP induced an extra drop in body weight occurring during the first five days after treatment (Fig. 2A). This treatment-induced body weight loss was additional to the disease-induced body weight drop and could be reproduced in healthy rats (data not shown). Body weight loss as a result of i.v. GC has been earlier reported for rats (Kaur et al., 1989; Rauchhaus et al., 2009b) and can be considered as a relevant parameter for systemic adverse events. As in our study the treatment-induced body weight fall was also seen in the case of

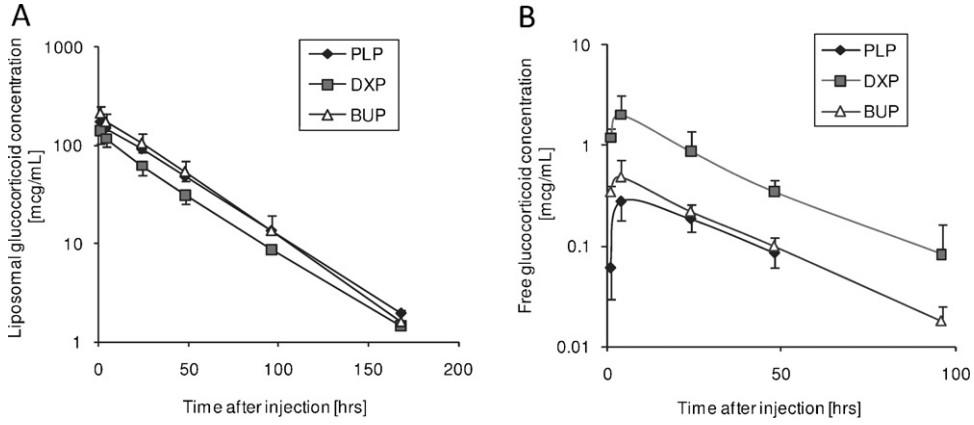

Fig. 5. Plasma concentrations of liposomal glucocorticoid phosphate (A) and released free glucocorticoid (B) in the circulation upon injection of 10 mg/kg glucocorticoid phosphate–PEG–liposomes. Data represent means of 4 rats, vertical bars show SD.

administration of free DXP, it could be that liposomal DXP induced this adverse effect as a result of the presence of free DXP in the circulation.

Besides the effect on body weight also the effect on blood glucose levels of GC can be used as a parameter for systemic activity (Ogawa et al., 1992; Rauchhaus et al., 2009b). In this study, monitoring blood glucose levels during the course of the disease showed that both liposomal DXP and free DXP caused a limited, but significant hyperglycemia during the first days after treatment (Fig. 2B). This observation again points to the presence of free dexamethasone in the systemic circulation after injection of liposomal DXP. Interestingly, an equipotent dose of liposomal PLP did not result in significant systemic adverse effects. Instead of a treatment-induced body weight loss, a strong regain of body weight was revealed in the first week after treatment with 10 mg/kg liposomal PLP, which clearly corresponded with the remission of paw inflammation (Fig. 3). Furthermore, no significant rise of blood glucose concentration was revealed upon 10 mg/kg liposomal PLP (data not shown). These observations suggest that the fraction of the i.v. administered dose of liposomal GC-phosphate that becomes available in the circulation as free GC may be much lower with liposomal PLP than with liposomal DXP due to the higher clearance rate of PLP, or that DXP has a higher intrinsic toxicity compared to PLP.

To investigate this, the third objective of the study was to evaluate whether there is a relation between the clearance rate of the encapsulated GC-phosphate ester and the quantity of free parent drug that becomes available in the circulation after i.v. administration of liposome-encapsulated GC-phosphate. With a higher clearance rate of the circulating free drug, one would expect the quantity of GC present in the circulation in free form to be less. In rats, there is a clear difference between prednisolone and dexamethasone regarding their clearance rate. Our results showed that liposomal encapsulation of PLP results in a stronger improvement of the therapeutic index than liposomal encapsulation of DXP. The absence of systemic activity of liposomal PLP regarding body weight loss (Fig. 3B) and hyperglycemia (data not shown) may indeed be explained by the high clearance rate of free PLP in rats. However, this observation may only apply to the rat. In humans, the clearance rate of prednisolone from the circulation is quite similar to that of dexamethasone. To optimize the therapeutic index of i.v. liposomal GC in RA patients, other GC should be selected with high clearance rates after i.v. administration in humans without forming active metabolites. Therefore, we selected BUP. The results show that liposomal BUP is twice as effective as liposomal DXP in rat AA (Fig. 4A) while showing less systemic side effect (Fig. 4B).

Comparing the plasma concentration–time curves of liposomal BUP with liposomal DXP and liposomal PLP after i.v. injection of equal doses revealed identical profiles for all three liposomal GC. Since it was shown before that no leakage of PLP and DXP from liposomes occurred, such may also be assumed for liposomal BUP. In contrast, the sustained free drug levels after injection of the three liposomal GC formulations greatly differed from each other with an inverse relationship with the clearance rate of the GC in question (Fig. 5B). The observation that free budesonide levels were slightly higher than free prednisolone is in agreement with the slightly lower clearance rate of budesonide reported in rats (1.5 $L\,h^{-1}\,kg^{-1}$ as compared to 2.3 $L\,h^{-1}\,kg^{-1}$ for prednisolone) (Chanoine et al., 1991; Nichols et al., 1989). However, this does not reflect the human situation, as in humans the clearance rate of prednisolone is much lower than that of budesonide (Thummel and Shen, 2001; Schimmer and Parker, 2001), and therefore in the human situation the therapeutic index would be improved even further with liposomal BUP.

In conclusion, our data showed that the use of GC (dexamethasone, budesonide) of higher potency than prednisolone, increases the therapeutic activity of liposomal GC. However, sustained free GC levels were observed after injection of the 3 liposomal GC-phosphates, which showed an inverse relationship with the clearance rate of the GC used. As the sustained free GC levels can cause systemic side effects, this study stresses the importance of a high clearance rate of the free GC in question for achieving a maximal therapeutic index with liposomal GC. Therefore high-clearance GC, which until now are only applied in local treatment approaches, may be very useful for the development of novel, highly effective anti-inflammatory preparations for systemic treatment of inflammatory disorders.

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