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Lipid nanocarriers as drug delivery system for ibuprofen in pain treatment

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Abstract

Due to their small size, lipid nanocapsules (LNC) might be promising for an injectable as well as for an oral drug delivery system, providing both sufficient drug solubility avoiding vessel embolisation for the intravenous injection and a positive effect of drug absorption after oral administration. Biocompatible ibuprofen LNC were developed in a size range of around 50 nm with a new preparation method. Drug incorporation into LNC was successful to a high degree in all formulations tested (94–98%) and the in vitro drug release in phosphate buffer occurred within 24 h. Pharmacokinetic data were recorded in vivo from rats after intravenous or oral administration, while the antinociceptive efficiency of the LNC formulation was compared with ibuprofen solution by the tail flick test. The AUC and half-life of intravenously injected ibuprofen LNC were found to be 16 and 19%, respectively, higher than a simple drug solution, while the mean residence time was not changed. Oral administration of LNC showed an 18% increase of AUC and a 27% higher mean residence time. The antinociceptive effect was similar for oral administration, drug solution, and LNC at 30 min after administration, and was prolonged up to 4 h in the LNC group. The pain relief after intravenous administration was prolonged when administering LNC formulation for at least 2 h. A drug delivery system for intravenous administration of ibuprofen has been developed which exhibits sustained release properties by either oral or intravenous route and may be interesting in the treatment of postoperative pain.

Keywords: Nanocapsules; Nanoparticles; Pharmacokinetics; Biocompatibility; Antinociceptive effect; Ibuprofen

1. Introduction

An alternative to the use of opioids in pain treatment is of ongoing interest, since the latter show distinct, undesirable, adverse effects, such as sedation, respiratory depression, constipation, and development of tolerance. For such an application, the group of non-steroidal anti-inflammatory drugs seems to be most promising. Although oral administration of these drugs is currently state-of-the-art, a major drawback from the formulation point of view is that these drugs are mainly lipophilic and therefore unsuitable for injection. An injectable formulation, however, would be desirable as it permits an immediate antinociceptive

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effect. Some studies for injectable ibuprofen formulations made use of co-solvents such as ethanol, which are not desirable for parenteral use and, moreover, not applicable in the treatment of children's pain. Furthermore, such co-solvent formulations may risk precipitation inside the blood vessels after intravenous administration as described for other drugs (Aravanis, 1982; Wehlage, 1973). Injectable prodrugs such as propacetamol have been introduced into the market where the drug is bound to some hydrophilic carrier. However, allergy reactions were observed after contact with skin (Barbaud et al., 1995, 1997). Therefore, the treatment remained following an oral administration of non-opioid drugs. Among them, ibuprofen has been proven for its efficiency in postoperative pain treatment in a wide field of applications (Cooper, 1984; Slavic-Svircev et al., 1984; Bostrom et al., 1994; Romsing and Walther-Larsen, 1997).

Another problem of major importance is the fairly short duration of action, since ibuprofen plasma half-life of 1–3 h has been reported (Kantor, 1979). Therefore, two major aims have been followed, the development of a harmless injectable formulation for ibuprofen which can simultaneously sustain the release of the incorporated drug load.

Nanosized controlled drug delivery devices consisting mainly of nanoemulsions, liposomes and nanoparticles have been proposed recently for both, oral and intravenous administration of drugs. A major drawback after intravenous administration of the system is a distinct accumulation of such carrier systems in the mononuclear phagocytotic system provoking a nearly immediate drug loss. This problem has been partially solved by the so called 'stealth' colloidal carriers which reduce the opsonisation by carrier's surface 'pegylation' (Gabizon et al., 1994; Gref et al., 1994; Tobio et al., 2000; Moghimi and Hunter, 2001). The polyethylenglycol surface coating was found also to reduce the fate of nanoparticles in the presence of intestinal enzymes after oral administration (Tobio et al., 2000).

Recently, lipid nanocapsules (LNC) were developed by a phase inversion technique demonstrating properties of a sustained drug release system (Lamprecht et al., 2002). Moreover, they provide a sufficiently hydrophilic surface in order to allow prolonged circulation of the drug carrier (Cahouet et al., 2002).

In this project, LNC were characterised for their suitability as a drug delivery device for an ameliorated pain treatment with ibuprofen. After an in vitro characterisation, ibuprofen loaded LNC were examined for their antinociceptive efficiency in rats. The carrier system was analysed for its pharmacokinetic behaviour after intravenous or oral administration and the connections with the efficiency in pain mitigation were analysed. Ibuprofen in solution was administered under the same conditions as the comparable standard formulation.

2. Materials and Methods

2.1. Materials

Ibuprofen was purchased from Sigma (Steinheim, Germany), Labrafac® CC, i.e. medium chain triglycerides (C8-C10 fatty acids), was kindly provided by Gattefossé (Saint-Priest, France). Lipoïd® S75-3 (soybean lecithin), Solutol® HS15 (polyethylene glycol-660 hydroxystearate), and Cremophor® EL (polyethylene glycol-35 castor oil) were kind gifts from Lipoïd GmbH (Ludwigshafen, Germany) and BASF AG (Ludwigshafen, Germany), respectively. All other chemical reagents were obtained from Sigma (Steinheim, Germany) and Fisher Scientific (Elancourt, France) and were of analytical grade.

2.2. Nanocapsule preparation

The preparation of LNC was based on a phase inversion method that allows the preparation of very small nanocapsules by a thermal manipulation of an oil/water system (Heurtault et al., 2002). Briefly, 20, 100 or 200 mg ibuprofen were dissolved in 993, 913 or 813 mg, respectively, of the internal oily triglyceride phase prior to all preparation steps by ultrasonication for 15 min. The oil phase was mixed with 875 mg of Solutol® HS15, 45 mg of Lipoïd® S75-3, 45 mg of sodium chloride, and 3.012 g distilled water and heated under magnetic stirring up to 85 °C (until a distinct drop of conductivity occurs) ensuring that the phase inversion temperature is passed. The cooling step was then performed until an exact temperature of 55 °C was reached, again, completely passing the phase inversion zone. This cycle was

repeated twice before adding 5 ml of distilled water at 2 °C.

2.3. LNC particle size analysis and atomic force microscopy (AFM) imaging

The LNC were analysed for their size distribution by photon correlation spectroscopy using a Malvern Autosizer® 4700 (Malvern Instruments, SA; Worcestershire, UK) at a fixed angle of 90 °. The zeta potential was determined with a Coulter Delsa 440 (Coulter Scientific Instruments, Amherst, MA, USA). All batches were diluted with distilled water prior to analysis and were analysed in triplicate.

The characterisation of ibuprofen-loaded LNC was performed with a commercial AFM (Autoprobe cp, Park Scientific Instruments, Sunnyvale, USA). The AFM was only used in the contact mode. LNC samples were placed on a mica plate surface by dropping 20 μl of the suspension onto the surface and removing the water under reduced pressure.

2.4. Determination of in vitro drug release kinetics

For calculation of ibuprofen encapsulation efficiency, LNC were separated from supernatant using Centrisart C30 microcentrifuge filters (Sartorius, Goettingen, Germany). Ibuprofen concentration was measured in supernatant by HPLC and encapsulation efficiency calculated referring to initial amount weighted. One millilitre of drug-loaded LNC suspension was filled into a dialysis tube (size exclusion: 100 000 Da) and inserted in a 100-ml flask containing 50-ml phosphate buffer (pH 7.4) in a water bath at 37 °C under gentle magnetic stirring at 250 rpm. At appropriate intervals, 0.5 ml samples were withdrawn and assayed for drug release and replaced by 0.5 ml of fresh buffer. The amount of ibuprofen in the release medium was determined by high performance liquid chromatography (HPLC) with a derivation from an earlier described method (Adeyeye and Price, 1997). The set-up was as follows: RP-18 column (LiChrospher® 100, Merck, Darmstadt, Germany); eluent: acetonitrile:water:acetic acid 500:477:3; flow rate 0.8 ml min^{-1}. Samples of 50 μl were injected into the column. All measurements were performed in triplicate.

2.5. Pharmacokinetic studies

Experiments were carried out in compliance with the regulations of the committee for animal experiments of the University of Angers in line with the French legislation on animal experiments. Sprague–Dawley rats (250–320 g) were used in all animal studies which were performed with six animals in each group. Food and water were available ad lib. All rats were anaesthetised with isoflurane during intravenous injection, oral administration, and blood sampling.

In the experiments for the oral ibuprofen administration route, rats received either LNC formulations (20 mg ml^{-1} ibuprofen drug load) or a bolus of ibuprofen dissolved in triglycerides at a dose of 50 mg kg^{-1}. The administered dose for the pharmacokinetic studies was equivalent to those in the antinociceptive studies at 50 mg kg^{-1}. At this concentration, significant pain relief was assured for the differently treated groups.

Rats receiving an intravenous administration of ibuprofen were either treated with LNC ibuprofen formulation or an injectable ethanolic emulsifier formulation of ibuprofen. Injections were performed into the penis vein at a dose equivalent to 50 mg kg^{-1}. LNC formulations were injected as prepared since the addition of NaCl during the preparation step already provided an isotonic solution. The intravenous ibuprofen solution was prepared by dissolving ibuprofen in a mixture of ethanol and Cremophor® EL (1:1) and slowly diluted with physiological NaCl solution under magnetic stirring until a final drug concentration of 20 mg ml^{-1} was reached.

Blood specimens of 0.5 ml were collected from the jugular vein sampling at different predetermined time points (15, 30, 60, 120, 240 and 360 min) in tubes containing 20 μl of heparin (2500 IU ml^{-1}) as anticoagulant.

0.3 ml of plasma was diluted with 1 ml of 0.2 M phosphate buffer (pH 2.0) and 5 ml of diethyl ether was added. The mixture was shaken for 3 min, and centrifuged at 10 000 × g for 15 min. The ether layer was collected, and the aqueous layer extracted with 5 ml of ether. The ether layer was added to that obtained previously. The ether phase was evaporated to dryness, and the residue was dissolved in HPLC eluent and 50 μl aliquots were injected on to the HPLC. All

HPLC analyses were run under the set-up described above. Pharmacokinetic values such as the area under the curve (AUC), half-life, and mean residence time (MRT) were calculated with Kinetica 4.0 (InnaPhase Corporation, Philadelphia, USA).

2.6. Tail flick test

For antinociceptive characterisation the tail flick test was used with 12 animals per group. All rats received either LNC formulations (20 mg ml^{-1} ibuprofen drug load) or a bolus of ibuprofen dissolved in triglycerides at a dose of 50 mg kg^{-1}. The antinociceptive responses were determined by measuring the time required to respond to a radiating thermal stimulus. The rat was restrained so that the radiant heat source was focused onto the tail. An Apelex tail-flick analgesymeter model DS 20 Socrel (Bagneux, France) was used, and the cut-off time was set at 30 s. For each rat, three determinations were carried out. Animals were acclimatised to the cages about 30 min prior to all measurements. The tail-flick latency responses were expressed in seconds. The isoflurane anaesthesia had no effect on the tail flick test series after 10 min.

2.7. Statistical analysis

The results were expressed as mean values ± S.D. The Mann–Whitney–Wilcoxon *U*-test was used to investigate differences statistically when the number of animals in each group was relatively low. When analysis of normality and equal variance were passed, the Student's *t*-test was also applied to examine the significance of differences. In all cases $P < 0.05$ was considered to be significant.

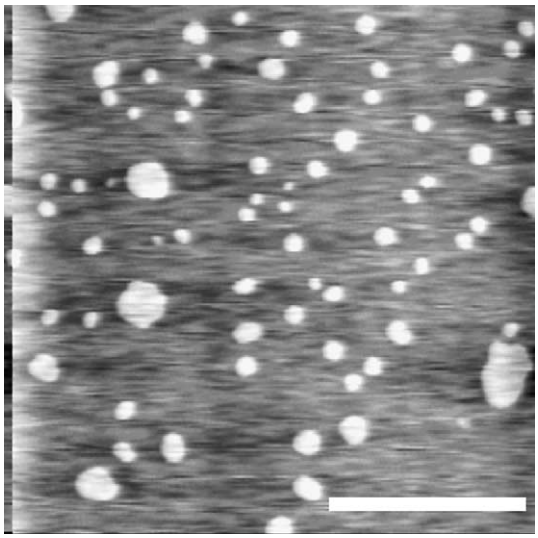

Fig. 1. Atomic force microscopic image of LNC of a nominal size of 50 nm. The scale bar represents 500 nm.

3. Results and discussion

3.1. Nanocapsule characteristics

Ibuprofen-loaded LNC prepared by the phase inversion processing had a submicron size and were relatively monodispersed as observed by photon correlation spectroscopy (Table 1). The particle size of LNC measured by this method was in the range of 45–60 nm. As reported in Table 1, the average size of the LNC capsules was mainly influenced by the amount of ibuprofen in the inner oil phase. Since LNC flattened during the drying step in sample preparation, the diameter observed by the AFM imaging procedure differed from the values of spectroscopical size determinations (Fig. 1). Similar in all batches, LNC had a

Table 1
Particle size, polydispersity, and encapsulation efficiencies of blank and drug-loaded nanocapsules

	PD (nm)	*P*	ZP (mV)	EE(%)
Blank LNC	57.0 ± 0.4	0.065 ± 0.008	−2.77 ± 0.36	–
Ibu LNC (2 mg ml^{-1})	56.5 ± 0.4	0.054 ± 0.008	0.91 ± 0.21	97.7 ± 1.3
Ibu LNC (10 mg ml^{-1})	52.8 ± 2.1	0.068 ± 0.014	0.46 ± 0.12	94.2 ± 3.6
Ibu LNC (20 mg ml^{-1})[a]	47.0 ± 0.3	0.094 ± 0.029	0.97 ± 0.32	96.4 ± 2.5

PD, mean particle diameter; *P*, polydispersity; ZP, zeta potential; EE encapsulation efficiency.
[a] Applied in the in vivo studies.

very low polydispersity index (0.03–0.06) and showed a monomodal particle size distribution, which qualified the carrier system for intravenous administration. Due to the low solubility of ibuprofen in the external aqueous phase during the preparation process, it was possible to reach very high encapsulation efficiencies varying between 94 and 98% of the initial ibuprofen amount which allowed an overall high drug load.

Fig. 2 illustrates the in vitro release kinetics of LNC in a phosphate buffer system at pH 7.4 at 37 °C. In the release pattern of all samples, an initial burst release was followed by sustained release indicating an entrapment of ibuprofen inside the lipophilic triglyceride core of the LNC. The drug release profiles achieved with different drug loading for ibuprofen showed a slightly accelerated release with lower drug content. The $t_{1/2}$-values were as follows: 2 mg ml^{-1}, 0.84 h; 10 mg ml^{-1}, 1.78 h; 20 mg ml^{-1}, 2.29 h.

By the high theoretical drug load a very efficient carrier system is afforded that can be achieved by the relatively low amount of required excipients compared to other nanoparticulate formulations when incorporating equivalent drug doses. Since no degradation was observed during in vitro release experiments, the drug release might mainly occur by diffusion throughout the lipid capsule matrix (Washington, 1996). The most appropriate LNC formulation for later in vivo characterisation was concluded to be the one providing the best prolonged release properties. Moreover, this system exhibited the most advantageous drug/excipient ratio for the different formulations at equivalent administered doses.

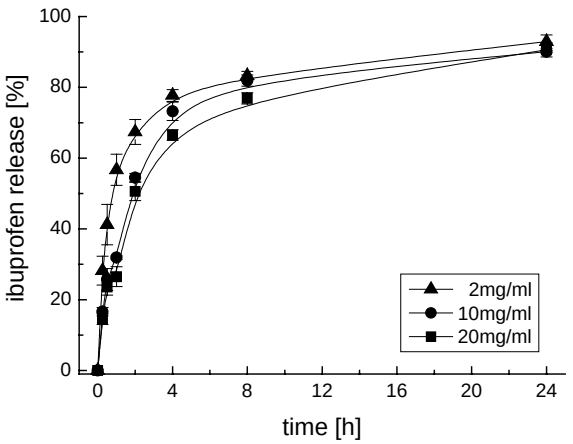

Fig. 2. In vitro drug release profiles for all batches in phosphate buffer (pH 7.4; 37 °C).

3.2. Oral LNC administration

In preliminary studies a constant dose of 50 mg kg^{-1} was adjusted to allow a distinct antinociceptive effect that was also in line with observations from earlier work (Price et al., 1996). A single oral administration of ibuprofen produced a clear increase of latency in tail withdrawal when measured 30 min after gavage (Fig. 3). Lower antinociceptive effects were observed after 30 min for ibuprofen LNC when given orally. However, effects were still significantly higher than the untreated control animals (all $P < 0.05$). Continuous observations after 2 and 4 h led to a distinctly decreased antinociceptive effect in the ibuprofen solution group similar to non-treated animals while the LNC group maintained a relatively increased level even at the final measurement.

The plasma concentrations after oral administration increased to their highest values after 30 min for both, free drug in solution and LNC preparations (Fig. 4). After 6 h, 96% of ibuprofen was found to be cleared from the plasma in all formulations. However, a prolonged presence of ibuprofen in the plasma was found in the LNC group, where the 1 and 2 h values were

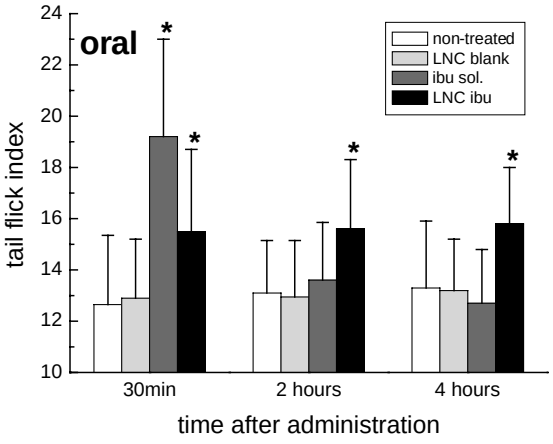

Fig. 3. Antinociceptive effects of ibuprofen solution vs. ibuprofen LNC formulation after oral administration. All tail flick test data are given as mean ± S.D. * $P < 0.05$ compared to untreated control rats given saline solution.

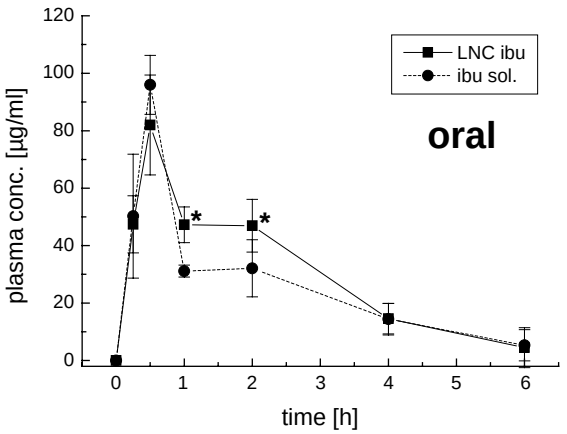

Fig. 4. Pharmacokinetic comparison of ibuprofen solution with ibuprofen LNC formulation after oral administration. $^{*}P < 0.05$ compared to the ibuprofen solution group.

significantly higher compared to ibuprofen solution. While for free ibuprofen an oral bioavailability of 86.8% was calculated, the AUC of LNC was about the factor 1.16 higher (Table 2). The half-life values of ibuprofen were reached at 72.8 min, meanwhile the LNC group was increased to around 18.8%. On the other hand, the MRT values between both groups did not differ significantly.

LNC exhibited a prolonged efficiency after oral administration compared to ibuprofen solution. This might be based on a sustained release via the oral route where consequently ibuprofen is not freely available for absorption from the intestinal lumen. Furthermore, it is reported that nanocarriers can be translocated across the gut wall (Florence et al., 1995) and in this case the entire LNC retain the controlled drug release properties, which may prolong the presence of the drug in the plasma. Besides, the transport across the mucosal barrier was found to be particle-size dependent (McClean et al., 1998; Hillyer and Albrecht, 2001) where the LNC diameter at around 50nm may exhibit relatively good prerequisites to be transported efficiently. However, an early drug release similar to that observed in the in vitro experiments may increase the bioavailability during the initial stage of the experiment. The sustained release properties of LNC by slowly delivering the entrapped drug with time and the prolonged therapeutic activity were found to be in contrast to the free drug which is immediately absorbed and eliminated.

3.3. Intravenous LNC administration

The prerequisite for an intravenously injectable drug carrier is its submicron size in order to avoid embolisation in capillaries where the smallest vessel diameters are estimated around 3 μm. Lipid nanocapsules seem to be suitable due to their monodispersed size distribution and the use of fully biocompatible excipients which received approval for use in humans. Moreover, this type of carrier has shown recently its prolonged presence in the blood circulation after intravenous injection (Cahouet et al., 2002).

When ibuprofen was injected intravenously, a similar initial antinociceptive effect was determined for both, simple ibuprofen solution and LNC (Fig. 5). The ibuprofen solution decreased in its activity; no significant differences to the control groups were found at the 2-h determinations. For the injected LNC slightly prolonged pain relief was observed. When administering ibuprofen LNC, the fade of effect was retarded and after 2 h significant pain reduction can still be achieved.

Generally, the plasma levels of ibuprofen LNC after intravenous injection were slightly increased compared to the ibuprofen solution group. During the first hour a slightly higher concentration was found for the free drug, while after 1 h the inverse tendency was continuously determined (Fig. 6). After 6 h, the remaining concentration of ibuprofen in the plasma was below 5% of the total administered dose. The AUC of

Table 2
Pharmacokinetic parameters in the different groups

	Ibu oral	Ibu LNC oral	Ibu i.v.	Ibu LNC i.v.
AUC ($\mu g\,ml^{-1}\,min^{-1}$)	10,329 ± 834	11,971 ± 713*	11,785 ± 1591	13,893 ± 1239*
MRT (min)	159.5 ± 26.6	157.2 ± 22.8	102.7 ± 6.2	130.7 ± 17.7*
$t_{1/2}$ (min)	79.8 ± 17.9	94.8 ± 13.6	72.8 ± 5.8	94.8 ± 13.0*

* $P < 0.05$ compared to non-LNC formulation.

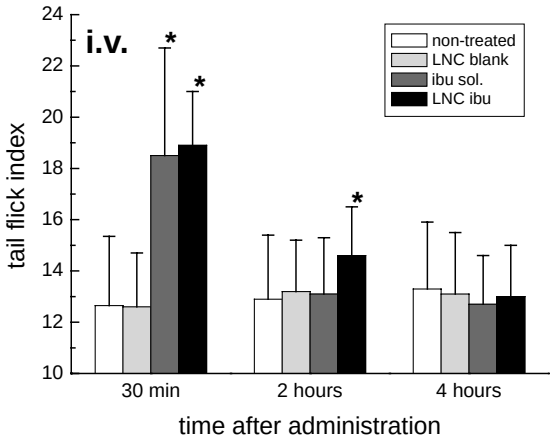

Fig. 5. Antinociceptive effects of ibuprofen solution vs. ibuprofen LNC formulation after intravenous administration. All tail flick test data are given as mean ± S.D. $^{*}P < 0.05$ compared to untreated control rats given saline solution.

the LNC formulation was significantly higher (18%) than when ibuprofen was administered in solution, and MRT and half-life were similarly increased by around 27% for the LNC compared to the ibuprofen solution. At $t < 1$ h, the free drug group exhibited the higher plasma level, which could be mainly based on the retention of drug inside the LNC and therefore retained from kidney filtration and elimination. The initial antinociceptive effect was mainly based on the early ibuprofen release similar to the findings after oral administration. Controlled drug release seems to be more likely than nanocapsule degradation, since LNC exhibited a sufficient ex vivo stability in blood (data not shown). The sustained release behaviour of the ibuprofen LNC ensured an antinociceptive effect after 2 h, which was not available for the injection of ibuprofen control solution.

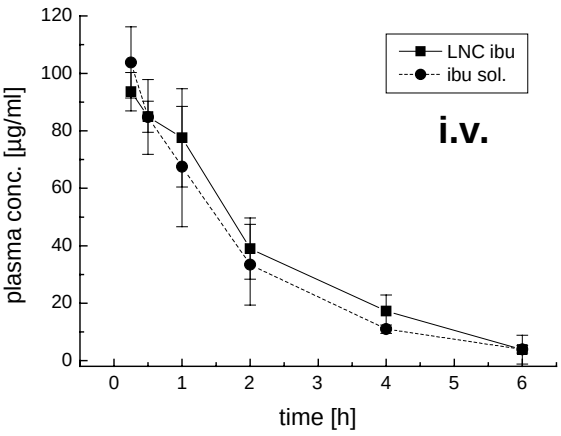

Fig. 6. Pharmacokinetic comparison of ibuprofen solution with ibuprofen LNC formulation after intravenous administration. $^{*}P < 0.05$ compared to the ibuprofen solution group.

The intravenous administration of ibuprofen solution included the use of ethanol and caused regularly tissue necrosis at the injection site. The lack of alternatives for injectable ibuprofen systems led to the ethanol formulation, proposed in several studies, which is, however, not applicable in clinical use. The LNC, however, provide a colloidal system which allows the intravenous administration of ibuprofen due to the low toxicity and no incidents were observed. Even if one considers the sustained release properties as minor, the development of a biocompatible, injectable formulation is still an important effort.

The prolonged efficiency of the LNC also seems to be dependent on their longer circulation period in the blood stream due to the hydrophilic surface of poly(ethylene glycol) moieties. These 'stealth' properties might be mainly contributed by the surfactant used in the preparation process constituting a surrounding layer that reduces opsonisation and therefore prolongs circulation time (Gref et al., 1994; Leroux et al., 1995). Early uptake into the mononuclear phagocytotic system can therefore be reduced and a distinct accumulation of the nanocarriers in the liver followed by a distinct loss of activity decelerated. During the presence in the plasma, LNC continuously release ibuprofen and allow a prolonged antinociceptive effect as shown by the tail flick test.

4. Conclusions

The preparation of LNC by a phase inversion method allows the formulation of nanocarriers without the use of organic solvents while high ibuprofen loadings can be achieved. This together with their monodispersed submicron-sized structure makes the carrier system attractive for oral and, in particular, for intravenous administration. Generally, LNC provide the possibility for an oral and intravenously administered formulation for ibuprofen which could be useful in the treatment of postoperative pain. For both, oral

and intravenous administration the nanocarriers allow, by their sustained release properties, a higher AUC value as well as a prolonged antinociceptive effect. It might be stated, that controlled release tablets may have comparable performance compared with LNC administered by oral route.

However, the more interesting findings consist in the design of an injectable carrier of ibuprofen which offers an alternative to other formulation based on co-solvents and less biocompatible surfactants.

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