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Review article

Lipid nanoparticles for parenteral delivery of actives

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ABSTRACT

The present review compiles the applications of lipid nanoparticles mainly solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and lipid drug conjugates (LDC) in parenteral delivery of pharmaceutical actives. The attempts to incorporate anticancer agents, imaging agents, antiparasitics, antiarthritics, genes for transfection, agents for liver, cardiovascular and central nervous system targeting have been summarized. The utility of lipid nanoparticles as adjuvant has been discussed separately. A special focus of this review is on toxicity caused by these kinds of lipid nanoparticles with a glance on the fate of lipid nanoparticles after their parenteral delivery *in vivo* viz the protein adsorption patterns.

1. Introduction

Parenteral drug delivery took a major leap after successful development of the submicronic parenteral fat emulsion (Intralipid) in 1960s. Quick commercialization of submicron emulsion-based products, such as Diazemuls (Diazepam) and Diprivan (Propofol), was the indicator of the interest of pharmaceutical industry in colloidal carriers. Since then, there have been continuous efforts to develop novel colloidal nanocarriers for improved parenteral delivery. The concept of lipid nanoparticles for injectable delivery was developed from submicron sized parenteral fat o/w emulsion used for parenteral nutrition viz Intralipid® in 1960s [1]. This gave birth to the idea of encapsulating lipophilic drugs into oil droplets. Products such as Diazemuls® contain diazepam and Diprivan® contain propofol [2]. The only drawback associated with these submicron emulsions was the low viscosity of the droplets causing fast release and susceptibility of the incorporated actives towards degradation by the aqueous continuous phase [3].

Liposomes represent the first generation of the novel colloidal carriers, which revolutionized the scenario in parenteral drug delivery. Liposomes offered several advantages such as encapsulation of hydrophobic and hydrophilic drugs, controlled drug release and reduction in toxicity/increased therapeutic efficacy of drugs most of which were not offered by submicronic emulsions. The successful commercialization ff various injectable liposomal products such as AmBisome® (Amphotericin B), Doxi®/Caelyx® (Doxorubicin) [6] and DaunoXome® (Daunorubicin) [7] and a large array of investigational products clearly indicates the potential advantages of liposomes as novel lipid carriers. However, complexity associated with the manufacturing of liposomes, difficulties in scale-up, limited physical stability and enormous cost of the liposomal formulation are the major barriers in the successful commercialization of liposomes [5].

Polymeric nanoparticles are also included in the first generation novel colloidal carriers developed with the objective to improve parenteral delivery. The particulate nature of polymeric nanoparticles, their ability to control the release of drugs and amenability for surface modifications were the drivers for active research on these carriers. However, due to various disadvantages such as difficulties in scale-up, high cost of biodegradable polymers, potentially toxic/allergic end products of biodegradable polymers [4], there is no commercial product based on polymeric 'nano' particles even after 35 years of their discovery and the first description of polymeric nanocapsules by Speiser and Birenbach. Nevertheless, polymeric 'micro' particle-based depot formulations are available in the market, e.g., Lupron® containing leuprolide and Parlodel® containing bromocriptin [5].

In 1990s, researchers (Mueller and coworkers and Gasco and coworkers) started exploring the potential of nanoparticles-based solid lipids or solid lipid nanoparticles (SLN) in the drug delivery. SLN are colloidal particles composed of a biocompatible/biodegradable lipid matrix that is solid at body temperature and exhibit size range in between 100 and 400 nm. SLN combine advantages of aforementioned colloidal drug carrier systems like liposomes, polymeric nanoparticles and emulsions, but at the same time avoid or minimize the drawbacks associated with them [8]. The various advantages such as particulate nature of SLN, amenability to encap-

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sulate hydrophilic and hydrophobic drugs, ability to sustain the release of incorporated drug, ability to prevent chemical, photochemical or oxidative degradation of drug, ability to immobilize drug in the solid matrix, ease of scale-up and manufacture and low cost of solid lipids as compared to phospholipids and biodegradable polymers give SLN an edge over aforementioned colloidal carriers [8].

Solid lipid nanoparticles (SLN) are colloidal particles of a lipid matrix that is solid at body temperature. They were first introduced by Müller et al. in 1993 [9,10] produced by high pressure homogenization and in parallel by Gasco by diluting warm microemulsion [11]. SLN have been exploited for delivery of actives via the dermal [12–14], peroral [15], parenteral [8,16], ocular [17–19], pulmonary [20–22] and rectal [23,24] route. Upon administration of SLN via the parenteral route of administration, improved bioavailability, targeting, enhanced cytotoxicity against multidrug resistant cancer cells have been observed.

Nanostructured lipid carriers (NLC) are composed of binary mixture of solid lipid and a spatially different liquid lipid as the carrier [12,14]. The major advantage of NLC is increased drug load. By now NLC are mainly investigated for dermal application [12,14] with seldom investigations focused on the parenteral route [65,66].

Lipid drug conjugates (LDC) were developed especially for the hydrophilic drug molecules, wherein an insoluble drug–lipid conjugate bulk is synthetically prepared either by salt formation (e.g., with a fatty acid) or by covalent linking (e.g., to the esters or ethers) [8,25]. LDC bulk is then homogenized in the presence of a stabilizer in water using high pressure homogenization.

The production of lipid particles in micrometer size range was reported in the late 1950s and in the beginning of 1960s [26,27]. Recently, a review [28] on advances in lipid nanodispersions for parenteral drug delivery and targeting has been published, which focuses on nanoemulsions, nanosuspensions and polymeric micelles. The first report on the use of SLN for oral delivery is by Speiser who termed them as nanopellets [29]. As the science of SLN technology progressed, different methods of production for them were developed and stable formulations of SLN were discovered. Earlier, the utilization of SLN for parenteral drug delivery [8] with focus on the definition of lipid nanoparticles and their different types such as SLN, NLC, and LDC, their production techniques, scale-up feasibilities, stability of the incorporated drug, release and the biological and biopharmaceutical aspects have been reviewed. In this review, we have summarized the efforts made by different groups to incorporate the actives in lipid nanoparticles, and the success of the drug delivery system has achieved till date with the focus on parenteral route of administration. We will also be discussing the new Pathfinder® technology, which can be used to target nanoparticles to the desired organ by modifying the surface protein adsorption on them.

The injectable lipid nanoparticles that have been studied so far have been encapsulated with anticancer agents, imaging agents, anti-parkinsonism, antiHIV, antipsychotics, anti-rheumatoid arthritic agents, antiparasitics, antihypertensives and antibiotics as summarized in Table 1. We will discuss the results obtained after encapsulating these therapeutic agents in lipid nanoparticles cate-

Table 1
Overview of various actives incorporated in injectable lipid nanoparticles

Drug	Disease	Type of lipid nanoparticle	Route of administration	Reference
3′,5′-Dioctanoyl-5-fluoro-2′-deoxyuridine	Cancer	SLN	IV	[60]
3-Azido-3-deoxythymidine palmitate/ azidothymidine	AntiHIV	SLN	IV	[101]
5-FU	Cancer	SLN	IV	[34]
99mTc/188Re	Imaging agent	Nanocapsules	IV	[79]
Actarit	Rheumatoid Arthritis	SLN	IV	[97]
All trans retinoic acid	Cancer	SLN	IV	[50,58]
Beta-element	Cancer	SLN	IV	[59]
Bromocriptine	Anti-parkinsonism	SLN	IP	[87]
Camptothecin	Cancer	SLN	IV	[16]
CdSEe/ZnS	Imaging agent	QDs encapsulated in SLN	IV	[81]
Clozapine	Antipsychotic	SLN	ID	[85]
Dexamethasone acetate	Pulmonary disease	SLN	IV	[100]
Diminazene	Antitrypanosomal	LDC	-	[95]
DNA	Cancer	catinoic SLN	-	[68–72]
Doxorubicin	Cancer	Stealth and non-stealth SLN, SLN	IV	[35,36,42,53,54]
Etoposide	Cancer	SLN	IV/SC/IP	[47,48]
Idarubicin	Cancer	SLN	IV or ID	[56]
Iron oxide	Imaging agent	SLN	-	[78]
Magnetite	Imaging agent	SLN	-	[77]
Methotrexate	Cancer	LMBVs	IV	[67]
Mitoxantrone	Cancer	SLN	Local injection in breast Cancer tissue	[49]
Nitrendipine	Antihypertensive	SLN	IV or ID	[61,62]
Oxymatrine	Antihepatitis	SLN	IV	[74]
Paclitaxel	Cancer	SLN/Wax NP/sterically stabilized SLN	IV	[37–41]
Paclitaxel and doxorubicin	Cancer	SLN	–	[42,43]
Paclitaxel and doxorubicin and cholestryl butyrate	Cancer	SLN	–	[44]
Quinine dihydrochloride	Malaria	Transferring conjugated SLN	IV	[94,95]
Tamoxifen	Cancer	SLN	IV	[45,46]
Tashione II A	Vasodialator	SLN	IV	[91,92]
Temoxifen citrate	Cancer	SLN	IV	[46]
Temozolomide	Cancer	SLN	IV	[64]
Testosterone 125 I radiolabelled	Imaging agent	SLN	IV	[80]
Tobramycin	Antibiotic	SLN	IV or ID	[86]
Vinorelbine bitartate	Cancer	PEG-modified SLN	–	[51]

IV, intravenous; ID, intrdeodenum; IP, intraperitoneum; SC, subcutaneous.

gory wise and the sequence is based on the amount of work done in the literature. Of the various purposes of these investigations, enhanced AUC and MRT, enhanced anticancer efficacy, enhanced brain targeting and enhanced targeting to diseased organ/cell were the main observations.

2. Application of lipid nanoparticles for parenteral drug delivery

2.1. Lipid nanoparticles for treatment of cancer

Several anticancer agents have been encapsulated in lipid nanoparticles, and their *in vitro* and *in vivo* efficacy has been evaluated by suitable studies. By and large, SLN have been shown to improve the efficacy and residence time of the cytotoxic drugs with concomitant reduction in the side-effects associated with them. This section would summarize the major studies reported in the literature, and for the detailed description readers are requested to refer to reviews by Wong et al. and Shenoy et al. [30,31]. The salient features of SLN which make them a suitable carrier for antitumor drug delivery are their ability to encapsulate antitumor agents of diverse physiochemical properties, improved stability of the drug, less *in vitro* toxicity, enhanced drug efficacy and improved pharmacokinetics [30].

The first *in vivo* studies encapsulating anticancer agent was performed in the year 1999 by Yang et al. [16]. They have investigated the body distribution of an antitumor plant alkaloid, camptothecin (CA)-loaded SLN (CA-SLN) prepared by high pressure homogenization and administered intravenously to mice. The average particle size of the SLN was 197 nm. The concentration of camptothecin at different time intervals after IV administration of CA-SLN in comparison to camptothecin solution (CA-SOL) in mice is depicted in Fig. 1. These SLN demonstrated higher AUC and MRT (18-fold enhancement) compared to camptothecin solution and accumulation, especially in brain, heart and reticuloendothelial cells containing organs. The authors attribute this efficacy to their small size or association of SLN with lipid bilayers of red blood cells. The enhancement in MRT was attributed to coating of poloxamer on the surface of SLN and sustained release of camptothecin from lipid matrix. The higher concentration of camptothecin encapsulated SLN in brain was speculated due to transport of intact SLN through the blood-brain barrier by endocytosis and simple diffusion of camptothecin released from SLN as shown in Fig. 1.

Drug-free stealth and non-stealth SLN were prepared by Podio et al. [32] using microemulsion template technique. They were

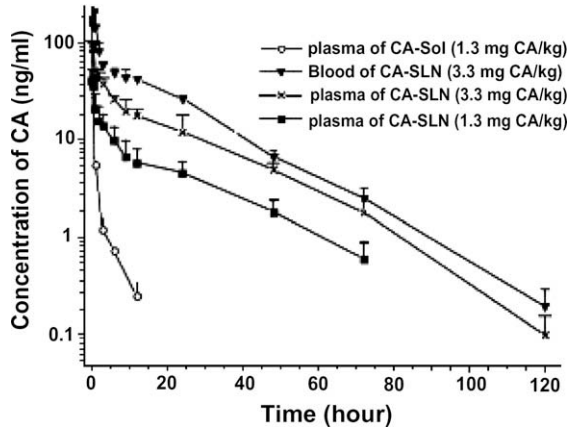

Fig. 1. Concentration–time curves of camptothecin after i.v. administration of CA-SLN with doses of 1.3 (■) and 3.3 (×) mg CA/kg in plasma and 3.3 (▼) mg CA/kg in blood, and CA-SOL with a dose of 1.3 (○) mg CA/kg in plasma. Results represent means ± SD of four animals. Reprinted with permission from [16].

injected intravenously in rats to study their tissue distribution and transport across the blood–brain barrier. The average size of these lipid nanoparticles was below 100 nm. These lipid nanoparticles were appropriately labelled using a radioactive marker. The results again confirmed localization of SLN in the brain and in the cerebrospinal fluid (CSF). This study also successfully demonstrated that SLN maintained their spherical shape and size in lymph and plasma after administration confirming their findings of earlier studies, wherein the SLN were prepared without any PEG coating [33].

Liver-targeted SLN containing 5-fluorouracil prodrug [34] were prepared by Yu et al., which were of average diameter of 240 nm. The mean content of these SLN in liver was 37.52% higher than in the control animal group, demonstrating selective targeting to liver by SLN.

Gasco and coworkers have investigated the potential of stealth and non-stealth SLN in improved parenteral delivery of doxorubicin [35,36]. In the first study [35], non-stealth and stealth SLN containing doxorubicin having average particle size 80 ± 5 nm (stealth) and 90 ± 5 nm (non-stealth) were prepared by microemulsion template technique. The pharmacokinetics and tissue distribution of doxorubicin in these SLN were studied after intravenous administration in rats and compared with commercial solution of doxorubicin. Prolonged circulation of up to 24 h was achieved. There was a significant increase ($p < 0.001$) in $t_{1/2}$, C_{max} (5-fold with non-stealth and 7-fold with stealth) and AUC values in stealth and non-stealth SLN loaded with doxorubicin as compared to commercial doxorubicin solution. There was low uptake of SLN in RES tissues, which was attributed to sizes and composition of SLN. Significant amount of doxorubicin was detectable in the brain and CSF of rats injected with SLN. Lower concentration of doxorubicin was found in heart when administered as SLN indicating lower cardiotoxicity compared to commercial doxorubicin solution, which is one of the effects limiting the usefulness of doxorubicin. In another study [36], non-stealth and stealth SLN (58–95.5 nm) encapsulating doxorubicin with increasing concentration of stearic acid-PEG 2000 as stealth agent were formulated by microemulsion template technique, and its pharmacokinetics and tissue distribution were studied in rabbits. Results indicate that the AUC of doxorubicin increased as a function of amount of stealth agent present in the SLN. Doxorubicin was detected in blood 6 hrs post-injection from both non-stealth and stealth SLN, while no doxorubicin was detectable after intravenous injection of doxorubicin solution. The highest concentration of doxorubicin in the blood was detected in stealth SLN with highest concentration of stealth agent. The results of this study corroborated the observations made in their earlier study in rats [36], where authors have attributed the brain targeting of stealth SLN to the retention of nanoparticles in brain–blood capillaries with absorption to capillary walls. This could create a higher concentration gradient leading to enhanced transport across the endothelial cells. Or else SLN may be endocytosed or transcytosed through the endothelial cell barrier, or SLN could also permeate the tight junctions between the endothelial cells.

Amongst various reports on potential of SLN in the parenteral delivery of anticancer agents, paclitaxel has been most extensively studied by researchers [37–41]. In the first study by Chen et al. [37], long circulating SLN were made from bioacceptable and biodegradable lipids from two types of stealth agents viz F68 (particle size 220 ± 98 nm) and Brij78 (particle size 103.5 ± 29.2 nm) by solvent emulsion injection evaporation method at low temperature. The *in vitro* release showed that the release for F68 SLN was slower than Brij 78 SLN, linear and followed Weibull equation. The pharmacokinetic studies in KM mice which were injected with these SLN revealed marked differences in pharmacokinetic parameters, especially in $t_{1/2}\beta$ and AUC, in comparison with Cremophore EL

containing solution of paclitaxel. F68 SLN and Brij 78-SLN were found to be long circulating with 7.4-fold and 3.6-fold higher $t_{1/2}\beta$, respectively. This was attributed to the reduced clearance rate and hence to the reduced uptake of SLN by mononuclear phagocytic system. Of the two stealth agents, SLN prepared with F68 were longer circulating than Brij 78 which was attributed to the longer PEG chain length.

In the studies by Koziara et al. [38,39], paclitaxel lipid nanoparticles having a particle size less than 100 nm were prepared by microemulsion template technique. In the first study [38], the potential of these SLN in the treatment of brain tumors was evaluated *in vitro*. They have tested the cytotoxicity of paclitaxel entrapped in novel cetyl alcohol/polysorbate SLN on human glioblastoma cell lines viz U-118 and HCT-15. The brain uptake of these SLN was evaluated using an *in situ* rat brain perfusion model. The results indicate that SLN increase the uptake in the brain and its cytotoxicity towards p-glycoprotein expressing tumor cells. In the other study by the same group [39], paclitaxel SLN prepared using emulsifying wax were tested *in vivo* in a HCT-15 mouse xenograft model. These SLN were demonstrated to overcome drug resistance in human colon adenocarcinoma cell line (HCT-15) and *in vivo* HCT-15 mouse xenograft model when injected intratumorally. In the results of the endothelial cell differentiation assays significant efficacy was achieved. The results of *in vivo* experiments are depicted in Fig. 2A and B.

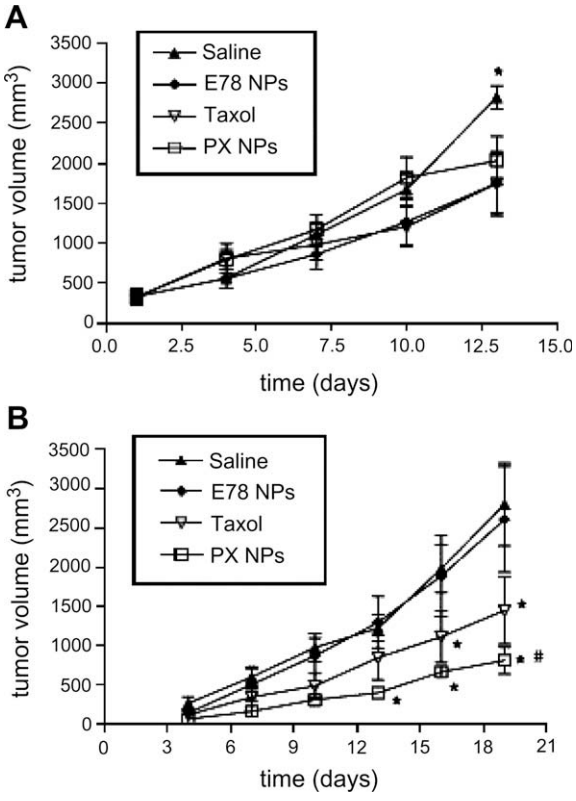

Fig. 2. Mean tumor volume as a function of time and treatment. (A) *In vivo* efficacy study #1 (study carried out 14 day after tumor implantation). Mice carrying HCT-15 tumors received direct intratumoral injections of saline, E78 NPs (blank nanoparticles incorporated in cetyl alcohol and stabilized by polysorbate), Taxol or PX NPs (Paclitaxel SLN) beginning 14 days after cell implantation and every 3 days for 13 days for a total of 5 injections. There were no significant differences in tumor volume between all the groups, with the exception of saline on day 13 ($^{*}p < 0.05$ between saline and remaining groups; two-way ANOVA with repeated measures, Fisher's LSD post-test). Data represent means ± SEM ($n = 6–7$). (B) *In vivo* efficacy study #2. Mice were dosed via direct intratumoral injections over the course of 19 days beginning 9 days after cell implantation. There was a significant inhibition of tumor growth in Taxol treated groups from controls ($^{*}p < 0.05$). Additionally, there was a significant difference between PX NPs and Taxol treatment on day 19 ($^{\#}p < 0.05$). Data represent means ± SEM ($n = 3–6$). Reprinted with permission from [39].

Sterically stabilized SLN [40] comprising of trymyristin and egg phosphotidylcholine and pegylated phospholipids as stabilizers and having average particle size around 200 nm were prepared using high pressure homogenization. The important finding of the study was that in the *in vitro* release studies SLN showed a slow but time-dependent release, and their *in vitro* cytotoxicities against human ovarian and breast cancer cell lines as determined by MTT assay were comparable to those of a commercially available cremophor EL-based paclitaxel formulation.

SLN loaded with doxorubicin or paclitaxel were found to have less cytotoxicity, and were found to be taken up by human promyelocytic leukemia (HL60) and human breast carcinoma (MCF-7) cells [42]. Similar results have been reported with HT-29 colorectal cancer cells too [43]. In a very recent study by Zhang et al. [44], the activity of nanostructured lipid carriers loaded with paclitaxel and doxorubicin was tested for cytotoxicities and reversal of drug resistance against different cell lines. The reversal power of NLC-loaded paclitaxel was 31 and for doxorubicin loaded NLC was 2.5 on testing it in a multi drug resistant cancer cell line (SKOV3-TR30).

Tamoxifen [45,46] is another anticancer agent that has been studied with injectable lipid nanoparticles owing to its application in breast cancer therapy. In a study by Fontana et al. [45], SLN were produced by two different production methods viz microemulsion templates and by precipitation technique with an average particle size of 118 and 69 nm, respectively. The *in vitro* antitumoral activity assay carried out on MCF-7 cell line (human breast cancer cells) suggests that tamoxifen SLN maintain an antitumoral activity comparable to free drug. The prolonged release exhibited by tamoxifen SLN indicates their usefulness in breast cancer therapy. The second study incorporated tamoxifen citrate in SLN [46], and studied the pharmacokinetic parameters of tamoxifen citrate-loaded SLN after intravenous administration in rats. The $t_{1/2}$ and mean residence time of these SLN in plasma were approximately 3.5-fold and 3-fold higher, respectively, than the free tamoxifen revealing their long circulating nature.

Tripalmitin SLN loaded with etoposide have been studied for biodistribution [47] and efficacy against Dalton's lymphoma tumor-bearing mice [48]. The biodistribution studies suggested that positively charged SLN have a high blood concentration and prolonged blood residence time and significantly lower uptake in reticuloendothelial system organs such as liver and spleen.

These positively charged SLN had 14-fold higher distribution in bone and brain than negatively charged SLN and etoposide 4 hours

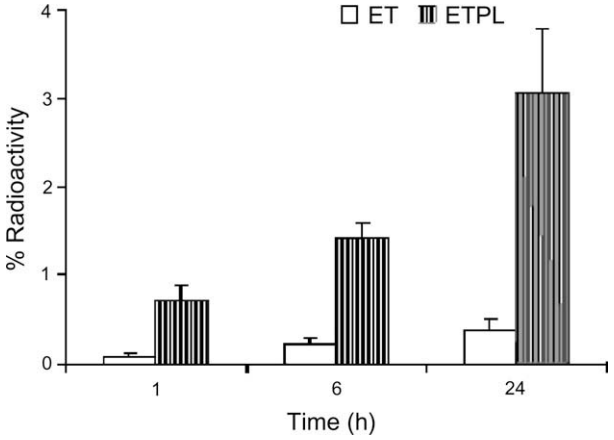

Fig. 3. Tumor concentrations of 99mTc-etoposide and 99mTc-ETPL nanoparticles after subcutaneous injection in Dalton's lymphoma tumor-bearing mice. Each value is the mean (FSD) of three experiments. ET, etoposide; ETPL, etoposide loaded tripalmitin nanoparticles. Reprinted with permission from [48].

after injection. The observed phenomenon was explained to be due to enhanced permeability and retention (EPR) effect. In the other study by the same group [48], these SLN were injected by various routes of administration like subcutaneous, intravenous or intraperitoneal, and their biodistribution and tumor uptake were determined, Fig. 3. The tissue distribution was found to be different for different routes of administration, and was found to be highest in the order of intravenous, intraperitoneal and subcutaneous administration. But the tumor uptake of etoposide SLN was 59-fold higher after subcutaneous injection in comparison with intravenous administration and 8-fold higher in comparison with intraperitoneal administration at 24-h post-injection.

A study [49] was carried out with local injection of mitoxantrone SLN against breast cancer and its lymph node metastases. It revealed that the drug concentration using SLN as the carriers was much higher in local lymph nodes, and the drug concentration in other tissue was lower than that of mitoxantrone solution. Moreover, there was no observed toxicity to the main tissues after local injection of SLN loaded with mitoxantrone compared to mitoxantrone solution. The percentage inhibition against breast cancer was 2-fold higher than that of mitoxantrone solution. The lymph node size of the mice after treatment with mitoxantrone SLN was approximately three times lower compared to treatment with mitoxantrone solution.

Another study was carried out on all-trans retinoic acid [50] in order to increase the chemical stability in powder form. The antiproliferative effects of SLN powder formulation tested on a range of cancer cell line were not significantly different from that of free all-trans retinoic acid. Moreover, its incorporation in SLN powder reduced the haemolytic potential.

An *in vitro* cellular uptake studies on vinorelbine bitartrate [51] encapsulated in PEG-modified SLN revealed that there was no phagocytosis of these SLN by RAW264.7 cells, but there was a significant improvement in the uptake by cancer cells (MCF-7 and A549) due to this PEG modification. Also the *in vitro* studies anticancer activity of vinorelbine bitartrate was found to be enhanced significantly after its incorporation in SLN and pegylated SLN.

SLN complexed with anionic polymer were formed to impart charge or enhance encapsulation, especially of water soluble. Such types of SLN have been used for encapsulation of drugs that have been studied for the delivery of cationic chemotherapeutic agents and chemosensitizers [52]. These polymer-complexed SLN had a particle size of around 180–300 nm. The efficacy of this system against multidrug resistant (MDR) cancer cells was determined [53]. These SLN were 8 times more efficient in killing the MDR cells compared to doxorubicin solution. The uptake and retention by MDR cells were both significantly enhanced by doxorubicin SLN. The authors have also evaluated the mechanism of overcoming MDR by these polymer lipid nanoparticles (PLN) [54]. Doxorubicin was found to be physically associated with SLN and can bypass the membrane associated Pgp (the cause of MDR) when delivered as doxorubicin PLN, the drug is better retained within Pgp-over expressing cells than the free drug.

PEG-coated gadolinium SLN were prepared using microemulsion templates [55], and were coated with folate. These SLN were found not to aggregate platelets or activate neutrophils. Both uncoated PEGylated and folate-coated PEGylated SLN were found to have enhanced cellular uptake and tumor retention. Higher amounts of folate-coated SLN, Fig. 4, were retained in the tumor tissue in comparison to PEG-coated SLN. The study suggests the potential of coated SLN in tumor-targeted delivery of gadolinium and therefore the increased therapeutic efficacy of neutron capture therapy.

Other literature examples of improving biodistribution and targeting effect of anticancer agents to brain following their administration as lipid nanoparticles are idarubicin [56], 4′-*O*-tetrahydropyranyl adramycin [57], 3′,5′-dioctanoyl-5-fluoro-2′-deoxyuridine [58], oridonin [59,60], aclaciomycin [61], and temozolomide [62].

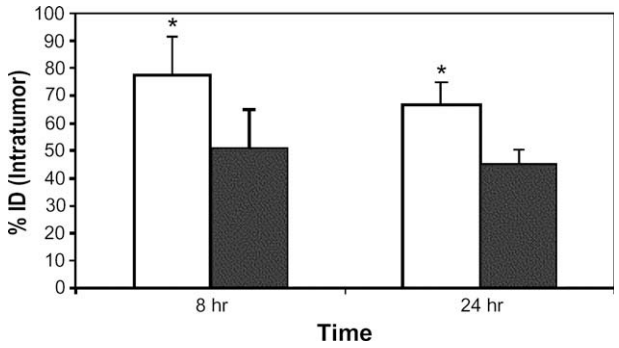

Fig. 4. Retention of folate-coated (empty bars) and PEG-coated nanoparticles (filled bar) after intratumor injection into KB tumor tissue developed in athymic mice. After 8 and 24 h, the mice were sacrificed and the amount of Gd NPs in the tumor tissue measured by a gamma counter. Each value represents means ± SD (n = 6–7 mice). (*Tumor retention of folate-coated nanoparticles was statistically higher than PEG-coated nanoparticles; $p < 0.02$; t-test.) Reprinted with permission from [55].

The second generation of lipid nanoparticles viz nanostructured lipid carriers have not been extensively studied as delivery systems for anticancer agents. In a study by Bondi et al. [63], NLC encapsulating two synthetic derivatives of antitumor drug temozolomide viz compound A and compound B were prepared. These NLC revealed an enhancement of the cytotoxic effects of compounds A and B on human prostate cancer (PC-3) and human hepatocellular carcinoma (HuH-6, HuH-7) cell lines with respect to free drug. In another study [64], encapsulating 9-nitrocamptothecin in NLC showed that stealth 9-nitrocamptothecin had sustained release characteristics and could resist the adsorption of plasma proteins to a certain extent. In the tissue distribution studies 9-nitrocamptothecin was mainly found in the lung, liver, pancreas, ovaries and uterus, and the AUC of 9-nitrocamptothecin-loaded NLC was higher than that of the solution. Also these NLC were shown to effectively target liver and lung.

An interesting type of SLN called as lipoprotein-mimicking biovectorized systems (LMBVs) was prepared for the delivery of methotrexate [65]. These LMBVs were prepared by microemulsion congealing technique, and palmitoylpolyethylene glycol 400 was anchored on LMBVs as apoprotein analogue. The pharmacokinetic studies carried out in rats suggested that the circulation half life of methotrexate was enhanced. Furthermore, methotrexate in LBMV was found to reside in tissues for a longer period of time as compared to that of control. These observations were attributed to the lipidic composition LMBVs and palmitoylpolyethylene glycol 400 anchoring which mimicks natural lipoproteins.

2.2. Lipid nanoparticles for transfection

The utilization of lipid nanoparticles for transfection has been documented in the literature [66–75]. Cationic SLN have been shown to be efficacious in transfecting COS-1 cells *in vitro* Olbrich et al. for the first time in the year 2001 [67]. These 100 nm SLN were able to bind DNA to form a stable complex of 300–800 nm size. The transfection efficacy as determined using COS-1 cells indicated that these cationic SLN complexes with DNA containing between 10 and 200 weight equivalents of SII 13 matrix lipid efficiently transfected galactosidase expression plasmid pCMγB in the presence and absence of endoosmolytic agent chloroquine as depicted in Fig. 5.

Tabatt et al. [68] have compared cationic SLN with liposomes for their transfection efficiency *in vitro*. The size of liposome was

found to be around 84 nm whereas that of the SLN was 148 nm. In the DNA binding efficacy and transfection studies both of them were found to be comparable. Also the same authors [69] have tried to optimize the formulation parameter of SLN viz the cationic detergent used and the lipid matrix used to enhance the transfection efficacy of SLN. The combination of acetylpalmitate as the lipid matrix and *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride as the cationic detergent resulted in highest transfection least toxic. The authors also found out that two-tailed cationic detergents are less toxic than one tailed ones.

The Mumper and coworkers have also investigated SLN for their transfection efficiency [70,71]. In one of the studies, emulsifying wax-based nanoparticles stabilized by cationic detergent cetyltrimethylammonium bromide (CTAB) were prepared from microemulsion templates, and they were coated with plasmid DNA on the surface. These SLN were also coated with mannan as a ligand to target dendritic cells. The ability of these SLN to increase the immune response was checked both *in vitro* by transfection efficacy studies and *in vivo* by measuring the immunization response after subcutaneous injection in mice. SLN resulted in 300% increase in cytokine production *in vitro* and 16-fold higher IgG titre and T-helper cells in comparison with the naked DNA. In another study by Cui et al. [71], two types of cationic surfactants viz DOTAP and DDAB were used to complex the plasmid DNA and encapsulated in the SLN prepared from emulsifying wax using microemulsion templates. They were also coated with pullulan, a hepatocyte targeting ligand. The *in vitro* transfection studies carried out in Hep G2 cells showed enhanced luciferase expression. Moreover, the *in vivo* studies carried out in mice demonstrated 40% transfection efficiency in case of SLN, which was significantly higher than that of naked DNA (16%).

As an approach for effective treatment of nasopharyngeal and prostate cancer, suicide gene therapy by local injection using folate-linked lipid nanoparticles has been used. This approach can effectively deliver genes extensively to FR-negative LNCaP and PC-3 as well as FR-positive KB and HeLa cells. This has been summarized in a review by Hattori et al. [72]. Specific surface receptor targeted DNA-SLN, which are stable under physiological conditions and low in cytotoxicity, and are capable of binding biotinylated ligands and interacting with surface receptors, have been investigated [75]. In their original work, [73] Bondi et al. [74] investigated cationic SLN as a non-viral transfection agent for gene delivery. These cationic SLN formed stable complexes with DNA and were found to protect DNA against DNAase I ligation. They

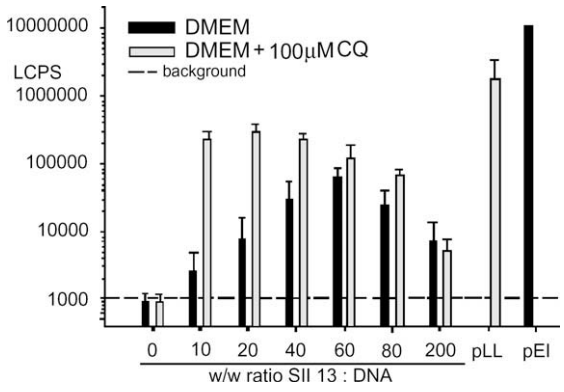

Fig. 5. Transfection efficacy of SLN (batch SII-13). Semiconfluent COS-1 cells were incubated for 4 h with transfection complexes in the absence and presence of chloroquine and analyzed after 48 h ($n = 6$). Reprinted with permission from [69] pLL, poly L-lysine; pEI, polyethylenimine (pEI); DMEM, Dulbecco's modified eagle's medium; CQ, chloroquine; LCPS, luminescence light counts per second; Batch SII-13 was composed of Compritol 4%, Tween 80/Span 85 (7:3) 4% and, *N*,*N*-di-(*b*-stearoylethyl)-*N*,*N*-dimethylammonium chloride (EQ1) 1%.

were found to have very low toxicity and were found to promote transfection of liver cancer cells.

2.3. Lipid nanoparticles for liver targeting

Particulate carriers (including SLN) usually accumulate in the liver by passive targeting on parenteral administration. However, passive targeting leads to entrapment of the drug in the Kupffer cells and not in the hepatocytes which is the major target for the treatment of hepatic diseases such as cancers. Hence, for liver targeting, SLN containing galactosylated or mannosylated lipids are employed. To date, there are very few studies which have systematically explored the liver targeting of SLN. Shen et al. [76] fabricated blank monostearin SLN with or without PEG 2000 modification and the pharmacokinetics of these radiolabelled SLN was studied in rats. The PEG-coated SLN showed 2.2 times longer circulation than that of unmodified SLN. However, unmodified SLN showed significantly higher accumulation in liver as compared to that of PEG-modified SLN. Oxymatrine, a hepatoprotective agent, was incorporated in SLN and its liver-targeting efficacy was determined in rats [77]. The mean content of oxymatrine in liver at 30 min was 12 times higher in SLN group than that of oxymatrine solution group. The relative-targeting efficiency to the liver tissues compared to solution was 360%. This targeting was achieved without any coating on SLN, and it could be attributed to the general clearance of nanoparticles by the phagocytic cells. Similarly, liver targeting of 5-fluorouracil prodrug has also been demonstrated by Yu et al. [34]. The 5-fluorouracil content in liver was 37.52% higher in case of SLN as compared to that of solution.

The utility of galactosylated excipients in the liver targeting has been investigated recently for an anticancer drug, taspine [78]. The SLN with and without galactosylation were evaluated for *in vitro* activity and *in vivo* biodistribution as compared to that of free drug. Both the SLN showed higher liver targeting as compared to that of free drug, and galactosylated SLN showed further enhancement in liver targeting.

2.4. Lipid nanoparticles for imaging

Magnetite was the first imaging agent which was incorporated in SLN [79]. The cytotoxicity of this magnetite-loaded SLN was compared with that of the magnetite-loaded polylactide/glycolide (PLA/GA) particles to determine toxicological acceptance as intravenous formulation for magnetic resonance imaging and as potential carrier for drug targeting. The magnetite-loaded SLN were found to be least cytotoxic with effective concentration ($ED_{50\%}$) above 10%, whereas that of the polymeric nanoparticles were found to be in the range of 0.15–0.38%. However, no *in vivo* studies have been carried out to validate *in vitro* observations.

First *in vivo* study incorporating iron oxide in SLN [80] was carried out in rats by Peira et al. Iron oxide SLN demonstrated similar relaxometric properties as that of Endorem®. *In vivo* magnetic resonance Imaging (MRI) of the central nervous system with both SLN and Endorem® showed that supermagnetic SLN have slower blood clearance than Endorem®. The retention in the CNS was found to be till the end of experiment that lasted for 135 min. These observations also boosted other findings like brain targeting by SLN. Intravenously injected ^{125}I radiolabelled SLN were found to remain in the blood for higher time in comparison to other colloidal carriers [81]. Luminescent lipophilic CdSe/ZnS core shell quantum dots (QDs) were encapsulated into SLN to prepare fluorescent nanocomposite particles [82]. The properties of QDs viz high fluorescence and narrow and symmetric emission spectra were retained even after encapsulation. Also due to encapsulation of several QDs into single nanoparticle structure, the fluorescence signal and the signal to background ratio were found to be enhanced. These QD-loaded

SLN were found to be stable, and the velocity of photobleaching was found to be reduced. The QDs were found to be biocompatible, and therefore have great potential in biological imaging.

2.5. Lipid nanoparticles for targeting the central nervous system

Various drugs ranging from antipsychotics, anti-parkinson, antieschemic to antibiotics have been encapsulated in lipid nanoparticles with the aim to either modify the biodistribution or for brain targeting [83,84]. Recently, a comprehensive review [85] covering various aspects of brain targeting using SLN has been published. Gasco and coworkers in a series of experiments evaluated biodistribution of radiolabelled non-stealth and stealth SLN after intravenous injection [32,33]. It was observed that stealth SLN circulated in plasma for a longer time. Furthermore, significantly higher concentrations of stealth SLN were observed in brain and cerebrospinal fluid. In another investigation, Gasco and coworkers evaluated biodistribution of tobramycin SLN after intravenous and intraduodenal administration [87]. Tobramycin-loaded SLN were found to cross the blood–brain barrier after intravenous administration which was significantly higher than intraduodenal administration.

Clozapine, a lipophilic antipsychotic drug, was encapsulated in various types of SLN, and its pharmacokinetic and biodistribution were studied on intravenous and intraduodenal administration [86]. Sterylamine-containing clozapine SLN were found to give significantly higher plasma levels and AUC as compared to clozapine SLN without sterylamine and clozapine suspension. Furthermore, biodistribution studies indicated that sterylamine containing clozapine SLN result in significantly higher amount of clozapine in brain (AUC) as compared to that of clozapine SLN without sterylamine and clozapine suspension. Furthermore, the mean residence time of these SLN was also higher than that of the other formulations.

Bromocriptine, an anti-parkinson agent, was encapsulated in NLC, and the *in vivo* efficacy of these formulations was tested. The *in vivo* efficacy of these lipidic nanoparticles was studied in 6-hydroxydopamine hemilesioned rats by giving intraperitoneal injection [88], which are models for Parkinson's disease. The results indicated that the anti-parkinsonian bromocriptin NLC have rapid onset of action as compared to that of solution. Furthermore, the anti-parkinson effect was retained for longer duration in case of NLC as compared to that of solution. This study indirectly demonstrated that higher brain levels of bromocriptine were achieved after administration as NLC. Suitable pharmacokinetic studies would be required to validate this observation. Cloricromene [89], which is used to reduce brain lipid peroxidation and the formation of post-ischemic brain edema, was encapsulated in SLN, and the *in vitro* release studies were carried out using human plasma. About 70% of drug was released within 15 min and immediately degraded by esterase to acid form, whereas after 4 h 30% of the drug remained entrapped in nanoparticles. Therefore, according to the authors SLN could be very useful for CNS targeting by intravenous administration. However, the speculations would have been more supportive if provided with *in vivo* data.

In a study by Chen et al. [90], dexamethasone-loaded SLN were administered by intratympanic (via inner ear) and intravenous routes. The AUC of dexamethasone acetate following intratympanic SLN administration was 13 times higher than the dexamethasone solution. Moreover, the AUC of dexamethasone in perilymph following intratympanic SLN administration was 76% lower compared to dexamethasone solution given by the same route. All these studies indicate great potential of lipid nanoparticles towards targeting the central nervous system and hence the brain.

Lockman et al. fabricated emulsifying wax and Brij 78 containing SLN. These SLN were further coated on surface with thiamine, and the brain uptake of the SLN with and without thiamine was studied using *in situ* rat brain perfusion technique [91]. A parameter called cerebrovascular permeability-surface area product (PA), which is an indicator of BBB uptake, was calculated and the results are depicted in Fig. 6. The coating with thiamine has been found to increase the unidirectional uptake transfer constant (k_{in}) to the BBB between 45 and 120 s. The thiamine-coated SLN showed significant potential in brain targeting.

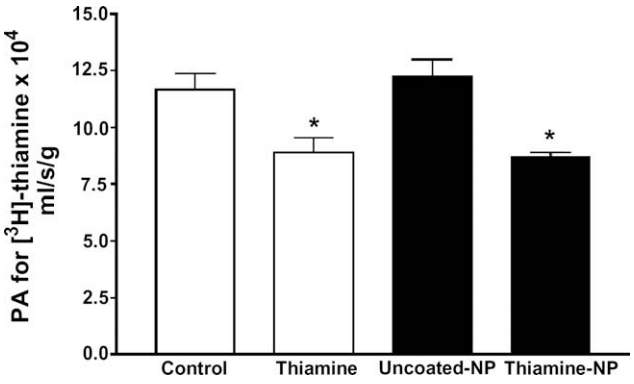

Fig. 6. Cerebrovascular permeability-surface area product of different treatments. An asterisk indicates that it differs significantly ($p < 0.05$) from control. Data are for frontal cerebral cortex and represent means ± SEM; $n = 3–5$. Reprinted with permission from [90]. The presence of SLN with thiamine ligand concentration of approximately 100 nM resulted in inhibition similar to the presence of 100 nM thiamine. The uncoated NP had no apparent inhibition of thiamine BBB transport. This indicates the association of thiamine ligand with thiamine BBB transporter.

Recently, potential of surface-modified SLN has been demonstrated in the treatment of brain diseases such as celebral malaria. Gupta et al. fabricated transferring-conjugated SLN and studied their ability to target quinine hydrochloride to brain by studying biodistribution [96]. The transferring-conjugated SLN showed enhanced tissue uptake in brain as compared to unmodified SLN and drug solution. Furthermore, biodistribution studies indicated that quinine hydrochloride concentration in brain was significantly higher in case of transferring-conjugated SLN as compared to that of unmodified SLN and quinine hydrochloride solution. To our knowledge, this is the first study which utilizes the principles of active targeting for the delivery of SLN.

All these studies indicate great potential of lipid nanoparticles towards targeting the central nervous system and hence the brain.

2.6. Lipid nanoparticles for the treatment of cardiovascular diseases

Tashinone IIA, a lipophilic natural drug product, has the ability to dilate coronary arteries and increase myocardial contractility. Liu and coworkers studied the ability of SLN to improve the delivery of Tanshinone II A by *in vitro* and *in vivo* studies. The poloxamer 188-coated SLN were fabricated, and the macrophage uptake of these SLN was studied in comparison to SLN without poloxamer 188. The poloxamer 188-coated SLN showed significantly lower macrophage uptake as compared to SLN without poloxamer coating [93]. The RES escaping ability of these poloxamer-coated SLN was evaluated by carrying out pharmacokinetic studies in rabbits. The pharmacokinetic studies [92] revealed that the area under the curve of plasma concentration time (AUC) and mean residence time (MRT) of the poloxamer-coated tashinone SLN were 3.3 times and 3.9 times higher than that of tashinone IIA control solution, respectively.

The pharmacokinetics of SLN loaded with nitrendipine, a calcium channel blocker used in the treatment of hypertension, were studied in male Wistar rats after intravenous and intraduodenal injection [94]. Effective bioavailability of nitrendipine-loaded SLN was 2.81- to 5.35-fold greater after intraduodenal administration

in comparison with that of nitrendipine suspension. In tested organs, the AUC and MRT of nitrendipine-loaded SLN were higher than those of nitrendipine suspension, especially in brain, heart and reticuloendothelial cells containing organs.

2.7. Lipid nanoparticles for treatment of parasitic diseases

Antiparasitic agents represent a class of drugs which had been neglected as a model for drug delivery systems for a long time. As compared to other therapeutic agents, relatively fewer reports are published on the delivery of antiparasitic agents. Recently, a comprehensive review dealing with the various drug delivery approaches for the treatment of parasitic diseases has been published [95]. There are few investigations which state the potential of lipid nanoparticles in the delivery of antiparasitic agents.

Transferring-conjugated SLN of quinine dihydrochloride [96], an antimalarial drug, were prepared to target it to the brain for the management of cerebral malaria. Enhanced uptake in brain tissues of transferring coupled SLN was observed in fluorescence studies. Also intravenous administration with these SLN resulted in much higher concentrations of drug in serum. Thus, this study again confirmed the utility of SLN in brain targeting. Another type of lipid nanoparticles which have been studied for the delivery of a hydrophilic antitrypanosimiatic drug diminazene [97] are the lipid–drug conjugates (LDC) nanoparticles. Here the hydrophilic drug, diminazene, was made lipophilic by conjugating it with stearic acid and oleic acid. From the lipophilic conjugate nanoparticles can be made by melting the conjugate (=lipid particle matrix material) and processing it identical to SLN by high pressure homogenization after pre dispersion in hot surfactant solution [25]. These nanoparticles were also shown to adsorb apolipoprotein E [98] after incubation with human serum, which is a key lipoprotein responsible for the delivery of nanoparticles to brain where the parasites *Trypanosoma brucei gambiense* and *rhodiense* reside. Recently, Joshi et al. [99] have explored the potential of NLC for intravenous delivery of an antimalarial drug, artemether. These NLC were found to be less haemolytic, and had significantly higher antimalarial activity compared to marketed intramuscular oily injection with significantly higher survival rate of 60% after 31 days of experiment.

2.8. Lipid Nanoparticles for treatment of rheumatoid arthritis

Actarit SLN [100] were prepared with the aim of passive targeting. These SLN were shown to enhance the therapeutic efficacy with concomitant reduction in the various adverse effects such as nephrotoxicity and gastrointestinal disorders. The pharmacokinetic studies in New Zealand rabbits revealed 10-fold higher mean retention time, 1.88 times higher area under curve (AUC) and around 3-fold increase in targeting efficiency with actarit-loaded SLN as compared to that of actarit solution upon intravenous injection.

2.9. Lipid nanoparticles for treatment of other diseases

Cholesteryl butyrate SLN [101] as a prodrug carrier for butyric acid have been prepared as an alternative to sodium butyrate in anti-inflammatory therapy of ulcerative colitis. The efficacy of cholesteryl butyrate SLN in inhibiting the adhesion of human neutrophils to endothelial cells has been studied in comparison with that of sodium butyrate. In all tests cholesteryl butyrate SLN were more active than sodium butyrate. Also cholesteryl butyrate SLN inhibited O_2^- production and myeloperoxidase release by polymorphonuclear cells in a dose and time-dependent manner. They were also found to be more active than sodium butyrate. From these observations, the authors concluded that cholesteryl butyrate-loaded SLN might be a better choice over sodium butyrate in the anti-inflammatory therapy of ulcerative colitis, which can avoid the complications related to sodium butyrate administration.

Glyceryl behenate lipid nanoparticles were injected endotracheally, and their lymphatic uptake was studied by radiolabelling [102]. It was observed that these nanoparticles are rapidly eliminated from rat lungs, and accumulation in para-aortic, axillary and inguinal lymph nodes starts almost immediately after administration. The translocation of nanoparticles across the lung mucosa and their uptake into the lymphatics have indicated their utility as drug carriers for lung cancer therapy and immunization process.

Dexamethasone acetate-loaded SLN [103] were administered by intravenous route of administration and were studied for biodistribution in mice. Post-injection the maximum level of dexamethasone was reached in 0.5 h. A 17.8-fold higher AUC was achieved using dexamethasone-loaded SLN as compared to dexamethasone solution. 3′-Azido-3′-deoxythymidine palmitate, an antiHIV prodrug, was encapsulated in PEG-modified SLN [104], and its biodistribution was studied by intravenous injection to CD-1 mice. The results suggest that there was an increase in bioavailability by encapsulation of 3′-azido-3′-deoxythymidine palmitate in SLN. SLN containing HIV protease inhibitor, atazanavir [105], was prepared, and its potential to deliver the brain microvessel endothelial cells was checked *in vitro* using hCMEC/D3 cell line. These SLN led to a significant accumulation in the brain endothelial cell as compared to the aqueous solution of drug.

3. Lipid nanoparticles as adjuvants

Lipid nanoparticles have been used as adjuvants for protein antigens and DNA for immunization. Olbrich et al. [106] have demonstrated the adjuvant activity of lipid nanoparticles using *mycoplasma bovis* antigen in sheep. Moderate activity was found in terms of antibody titre in comparison to Freud's incomplete adjuvant (FIA), which could be improved by combination with EQ1 (*N*,*N*-di-(β-stearoylethyl)-*N*,*N*-dimethyl-ammonium chloride). As against the FIA, the SLN-based antigen formulations were well tolerated. Further, our research group has also shown [107] that paraffin or biodegradable glycerides-based SLN containing *mycoplasma bovis* antigen and immunoglobulin G (IgG) would be a promising alternative to Freud's complete adjuvant (FCA). This was the first study which showed that the adjuvant activity of SLN is dependent on their particle size. Particles greater than 100 nm exhibited higher adjuvant activity as compared to the particles lower than 100 nm.

In another study by Cui and Mumper [108,109], a catonized model protein antigen was coated on anionic lipid nanoparticles. The cationized protein-coated nanoparticles yielded strongest and most reproducible antibody titre upon subcutenous injection to mice in comparison to cationized antigen alone or non-cationized antigen administered together with alum as an adjuvant. The lipid nanoparticles were found to enhance both T-helper type 1 and type 2 immune responses. Cui and Mumper [110] have also studied the effect of co-administration of adjuvants with lipid nanoparticles-based genetic vaccine delivery system on the immune response. They found significant enhancement in immunization over naked plasmid DNA, e.g., 300-fold for cholera toxin (100 μg) and 250-fold for lipid A (50 μg) by subcutaneous route.

4. *In vivo* fate of lipid nanoparticles: protein adsorption patterns

It is noteworthy that the *in vivo* fate of drug on parenteral administration is no longer determined by the properties of the drug but by the type of the drug delivery system used, which in this case are the lipid nanoparticles [111]. The mononuclear phagocyte system (MPS) plays a vital role in clearing the nanopar-

ticles from blood circulation. It has been shown by our research group [112–116] that the type and pattern of protein adsorption on lipid nanoparticles determines the organ distribution of lipid nanoparticles. Hence, it can be assumed that the targeting of lipid nanoparticles is dependent on their physiochemical properties. After intravenous injection, a protein gets adsorbed on the lipid nanoparticles surface depending upon their surface properties. The adsorbed protein leads to the adherence of these nanoparticles to cells with appropriate receptor on the surface. For example, particles having adsorbed proteins with opsonic function, e.g., immunoglobulin IgG, complement factor C4γ are cleared by MPS cells, whereas the absence of opsonins in the adsorption pattern and the presence of dysopsonins (e.g. Albumin, IgA) lead to the circulation of particles in the bloodstream. Coating of nanoparticles with apolipoprotein E leads to their preferential targeting to the brain as apolipoprotein E plays an important role in the transport of lipoprotein into the brain via the low density lipoprotein (LDL) receptor. Hence, it is likely that apolipoprotein E adsorbing drug carriers mimic lipoprotein particles, leading to brain uptake by endocytic processes.

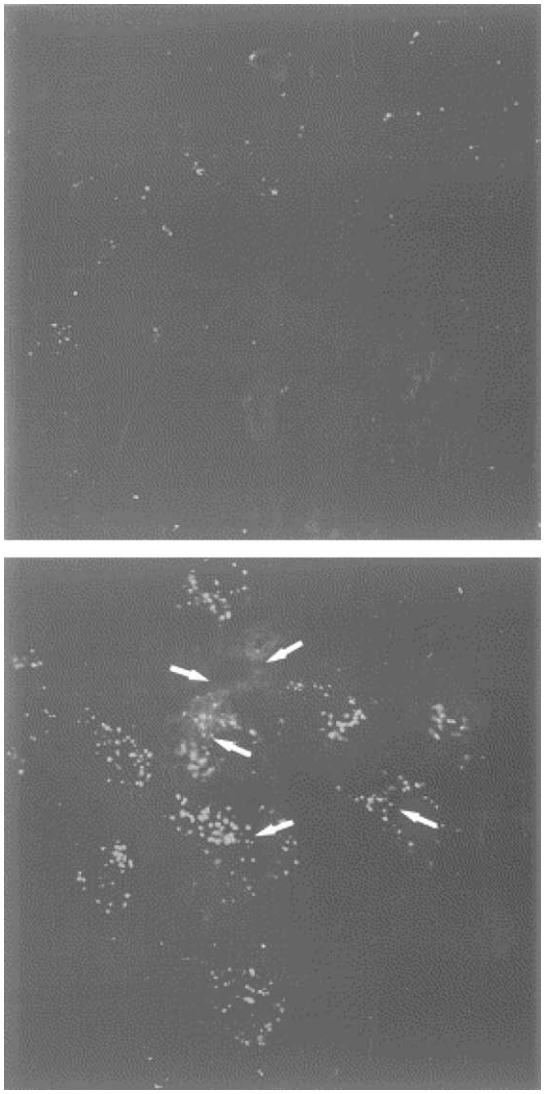

Fig. 7. Confocal laser scanning micrograph of mouse brain tissue, upper: control, lower: after i.v. injection of Nile Red-labelled LDC nanoparticles. Arrows indicate the nanoparticles adhering to the endothelial cells of the brain vessels and the diffusion of the dye into the brain tissue. Reprinted with permission from [121].

A two-dimensional polyacrylamide gel electrophoresis (2-DE) has been proven to be an effective tool for detecting all proteins adsorbed onto a nanoparticulate carrier. The technology named Pathfinder® [117,96] has been developed by our research group using the principle of differential protein adsorption. The Pathfinder® technology identifies the naturally occurring mechanism for localization of material in different parts of the body via adsorbed blood proteins. Controlled production of carriers with appropriate surface properties leads *in vivo* to preferential adsorption of the targeting protein and subsequently to site specific intravenous delivery.

Surface-modified particles which were able to deliver drugs to the brain have been reported by the Kreuter group [118–122]. It was confirmed by 2D-PAGE that the uptake of nanoparticles to the brain endothelial cells was mediated by apolipoprotein E. In further experiments, the negative control of this experiment was precoated with apolipoprotein E on the surface and it was found to target the brain, thus providing the proof of the principle. The Pathfinder technology was successfully implicated to target the brain using nanocrystals [123], lipid drug conjugate nanoparticles [124], but its utility to target blood–brain barrier (BBB) using NLC remains to be elucidated. The efficiency of apolipoprotein E coated LDC to selectively target brain as determined by fluorescence microscopy is depicted in Fig. 7.

To target nanoparticles to the brain, one can either modify the surface of nanoparticles so that they can selectively adsorb the protein of interest to target it to the organ of interest or the protein itself can be preferentially adsorbed on the particles prior to injection, alternatively the receptor binding moiety, e.g. class G amphiphilic helixes for apolipoprotein E, can be coupled covalently to the particle surface.

In a study by Göppert et al. [116], the influence of different surfactants on the *in vitro* adsorption of human plasma proteins was investigated using 2-DE. There was a correlation with different amounts of adsorbed apolipoprotein E and the hydrophilic lipophilic balance (HLB) of the different polysorbates used in the preparation of SLN.

5. Toxicity of lipid nanoparticles

For the successful regulatory clearance of SLN for parenteral delivery, it is essential to establish their biocompatibility with blood components and other tissues. Hence, to study the toxicity of lipid nanoparticles various *in vitro* and *in vivo* efforts would be discussed herein. The interaction of SLN and their respective cytotoxicities was studied with human granulocytes [125], HL60 cell line [126], RAW 264.7 macrophage [127] and murine peritoneal macrophages [128]. The experiments with the interaction of SLN with human granulocytes [125] revealed that SLN had distinctly lower uptake by phagocytosis resulting in prolonged circulation time in blood. Also the study pointed out the ~10-fold low cytotoxicity of glyceride SLN in comparison with that of polylactide/glycolide nanoparticles. Thus, SLN can be used as intravenous carriers because of their prolonged circulation time and high toxicological acceptance. The interaction of SLN with HL60 cells [126] gave similar results showing lower cytotoxicity compared to polymeric nanoparticles, whereas the interaction of Dynasan 114 SLN on RAW 264.7 macrophages [127] has revealed that those SLN stabilized with pharmaceutically acceptable surfactants like poloxamer 188, Lipoid S75, sodium cholate, Tween 80 are very well tolerated by RAW 264.7 macrophages. The tolerance was judged in terms of cytokine production, which was reduced and stimulation, expressed in elevated cytokine levels, could not be found. Similar results were obtained earlier when Dynasan 114 SLN were interacted with peritoneal mouse macrophages [129–131]. In a study by Schöler et al. [132], the behaviour of murine macrophages in the presence of different concentration and size and various concen-

trations of SLN was studied. SLN made from lipids consisting of stearic acid or dimethyl-dioctadecylammonium bromide were found to be cytotoxic at the concentration of 0.01%. On the other hand, SLN made from lipids consisting of triglycerides, cetylpalmitate or paraffin were found to be safe at the same concentration. Authors speculate that decrease in IL-6 production based on the concentration of the lipid could be the reason for such kind of cytotoxicity. However, the cytotoxicity was independent of the size of SLN. In an *in vivo* study by Weyhers et al. [128], a greater depth was achieved in understanding the toxicity created by SLN *in vivo* when two types of SLN, one made from Compritol (GRAS approved) and the other made from Cetyl palmitate (less physiologic), were administered six times within a period of 20 days via bolus injection. The injected doses were extremely high, i.e., up to 1 g of solid lipid per kg of body weight. In humans, this would be compared to bolus injection of 75 g of solid particles intravenously. The results of histopathology suggested that the toxicity is dependent on the lipid matrix as well as the dose administered despite this high dose. No untoward results were obtained with cetyl palmitate SLN, while the high dose compritol-containing formulation led to the accumulation of lipid in liver and spleen of mice and to subsequent pathological alterations. However, these alterations were reversible within six weeks after intravenous administration, which was attributed to the slow degradation of compritol. In *in vitro* degradation studies it could be shown that cetyl palmitate was degraded much faster than compritol corroborating the results of *in vivo* study. By reducing the compritol dose to 0.25 g, still being a high dose, all these observed alterations did not occur any more. In another study by Heydenreich et al. [133], cationic SLN containing sterylamine and different triglycerides were prepared and purified using different methods such as ultrafiltration, ultracentrifugation and dialysis. As it is suspected that toxicity of SLN is dependent on the composition and the purification method used, their cellular toxicity and physical stability were compared. The cell toxicity was found to be dependent on the composition of SLN and the method of purification used. Dialysis was found to be the most efficient to remove excess surfactant. Also, a correlation could be obtained on the basis of the amount of sterylamine to toxicity produced which was not observed in the case of liposome.

6. Conclusion

The potential of lipid nanoparticles for the parenteral delivery of various therapeutic agents has been successfully established. The successful fabrication of transferring-conjugated SLN has opened a new era for active targeting using SLN, although extensive studies are required to be carried out. The parenteral acceptability of the lipids and other surfactants is the major hurdle in their successful commercialization. It is possible to use glycerides of the fatty acids which are present in the lipid phase of parenteral fat emulsions (e.g., C14-C18 or C22 fatty acids) to fabricate SLN with high biocompatibility [134,135]. However, extensive *in vivo* studies are required to establish their *in vivo* parenteral acceptability. The other aspects of SLN such as sterilization [136], freeze drying [137,138], shelf life [139,140] and large-scale industrial production [139] have already been developed to a sufficient standard with the view of commercialization. Thus, although there is no lipid nanoparticle-based injectable product in the market till date, with the progress which they have seen so far, the day for the marketed arrival of SLN might not be too far.

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