Department of Pharmaceutics, Biopharmaceutics and Biotechnology[1], Department of Medical Microbiology and Immunology of Infectious Diseases[2], Free University of Berlin, Berlin, Germany

Solid Lipid Nanoparticles (SLN) – Effects of lipid composition on *in vitro* degradation and *in vivo* toxicity

H. Weyhers[1], S. Ehlers[2], H. Hahn[2], E. B. Souto[1], R. H. Müller[1]

Received August 5, 2005, accepted August 23, 2005

Prof. Dr. R. H. Müller, Department of Pharmaceutics, Biopharmaceutics and Biotechnology, Free University of Berlin, Kelchstraße 31, D-12169 Berlin, Germany
mpharma@zedat.fu-berlin.de

Pharmazie 61: 539–544 (2006)

Solid lipid nanoparticles (SLN) composed of two different lipid matrices were produced to assess their *in vivo* toxicity in mice. Matrix substances were (i) Compritol (glycerol behenate), a physiological lipid with GRAS status (generally recognized as safe [FDA]), and (ii) cetyl palmitate, a less physiological compound. Physicochemical data proved the suitability of SLN batches for intravenous administration. To assess the *in vivo* toxicity of produced batches, 400 μl SLN dispersion (lipid content 10% [m/m]) were administered to mice via a bolus injection for six times within a period of 20 days (high dose administration). Additionally, a multiple low dose administration was performed with Compritol-SLN as well (200 μl SLN dispersion, lipid content 2.5% [m/m]). Hepatic and splenic tissues were analysed histologically. *In vivo* results were dependent on the lipid matrix, as well as on the dose administered. For cetyl palmitate containing SLN no pathological results were obtained, while high dosed Compritol containing formulations led to accumulation of the lipid in liver and spleen and subsequently to pathological alterations. These alterations were found to be partially reversible within six weeks after completing intravenous administration. Liver architecture was nearly recovered. In contrast, low dosed Compritol SLN were well tolerated. Lipid accumulation and pathological alterations of high dosed Compritol SLN were attributed to the slow degradation of the Compritol matrix which could be shown by performing *in vitro* studies in human plasma.

1. Introduction

In 1991 solid lipid nanoparticles (SLN) were introduced as an alternative colloidal drug carrier for oral, parenteral and topical use (Müller and Lucks 1991; Müller et al. 2000a). SLN combine advantages of polymeric nanoparticles and fat emulsions for parenteral nutrition (Müller et al. 1995, 2000a; Mehnert and Mäder 2001). Similar to polymeric nanoparticles SLN possess a solid biodegradable matrix. Solid matrices allow a controlled modification of the release profile of incorporated drugs. Similar to emulsions and liposomes they are composed of physiological lipids (e.g. triacylglyerols) or lipoids and can be produced on industrial scale using high pressure homogenization technology (Müller et al. 2000b; Müller and Souto 2005). Simultaneously, the major disadvantages of polymeric particles (use of organic solvents in production, cytotoxicity of monomers, lack of large scale production technology) and of fat emulsions (burst release of incorporated drugs) can be eliminated (Müller et al. 1995, 2000c).

Optimized SLN dispersions show a negligible content of particles above 5 μm. Therefore, intravenous administration of SLN is possible (Schwarz et al. 1994a). For optimized aqueous SLN dispersions a long-term stability of at least 3 years was shown (Freitas et al. 1994). Alternatively, SLN formulations can be lyophilised or sprayed-dried (Schwarz et al. 1994b; Schwarz and Mehnert 1997). Autoclaving or gamma irradiation was found to be suitable for sterilisation of aqueous dispersions. Lipophilic, as well as hydrophilic or insoluble drugs, can be incorporated in SLN (Müller and Souto 2005). These carriers were shown to have controlled release properties (Müller et al. 2000b) for several drugs, such as prednisolone (Müller et al. 1994), retinoids (Jenning et al. 2000) and clotrimazole (Souto et al. 2004). *In vitro* toxicity studies of SLN showed less cytotoxicity compared to nanoparticles composed of polylactide/glycolide (PLGA) (Müller et al. 1996a, b).

From these features, SLN appear as an alternative colloidal carrier system. However, prior to its use as carrier system, there is a need for a quantification for the *in vivo* toxicity of SLN administered intravenously. Many data are available of liquid lipids administered as o/w emulsions intended for parenteral nutrition, but little is available concerning the i.v. injection of solid lipids as nanoparticulate suspension. In this study, two chemically different lipids – a glyceride composed of physiological behenic acid (Compritol ATO 888) and a wax (cetyl palmitate) – are investigated to assess their *in vivo* tolerability after multiple high i.v. dosing.

Table: PCS and laser diffractometry analysis of SLN

SLN formulation Lipid content	Compritol 10% [m/m]	Compritol LD 2.5% [m/m]	Cetyl palmitate 10% [m/m]
PCS diameter (nm)	239.1	238.4	254.4
PI	0.185	0.176	0.141
LD analysis			
99% (μm)	1.23	n.d.	1.67
95% (μm)	0.89	n.d.	0.97
90% (μm)	0.74	n.d.	0.77
50% (μm)	0.36	n.d.	0.35

(PI, polydispersity index; n.d., not done; D99%, volume distribution of 99% of the particles that is smaller than the listed values)

2. Investigations and results

2.1. Particle size analysis

The mean diameter (PCS data) of the Compritol batch (lipid content 10% [m/m] was 239 nm, and 265 nm for the cetyl palmitate containing batch (lipid content 10% [m/m]). Polydispersity indices (PI) were found to be 0.19 and 0.14, respectively. Due to the fact that the Compritol LD batch (content of lipid reduced to 2.5% [m/m]) was prepared by diluting the 10% Compritol batch, the data obtained for this formulation did not differ significantly (Compritol LD batch: 238 nm, PI: 0.18). Therefore, further physicochemical investigations (analysis by laser diffractometry, LD) were only performed for the high concentrated Compritol stock. LD analysis was performed to evaluate a possible contamination of the produced batches by microparticles. Results are shown in the Table. The diameters D99%, D95% and D90% based on the volume distribution of the particles are listed. These parameters (D99%, D95% and D90%) were found to be most sensitive for the determination of particle aggregates (Bock 1994). D99% values of about 1.7 μm and 1.2 μm were found for the Compritol SLN and for the cetyl palmitate SLN (lipid content 10% [m/m] for both), respectively. To assess the suitability of the SLN for i.v. administration, the results were compared with data obtained form parenteral fat emulsions. Mean diameters (PCS data) in the range of 250–400 nm are typical for fat emulsions (Müller and Heinemann 1992). LD data are also available for fat emulsions for parenteral use: D99% values in the range of 0.99 μm up to 2.19 μm are published depending on the commercial product (Bock 1994). To quantify the microparticular contamination in terms of numbers per volume unit, Coulter Counter measurements were performed. Results are shown in Fig. 1. The obtained results were compared to data of commercial fat emulsions for parenteral use (Intralipid™, Lipofundin™). For these emulsions the content of particles above 5 μm were found to be in the range of 348 up to 1473 particles per μl emulsion (Schwarz et al. 1994a). The prepared SLN dispersions showed the following results: 140 particles per μl Compritol SLN dispersion and 349 particles per μl cetyl palmitate SLN dispersion (Fig. 1). Considering these results, the produced SLN batches were of similar quality as parenteral emulsions and were therefore regarded as suitable for intravenous administration.

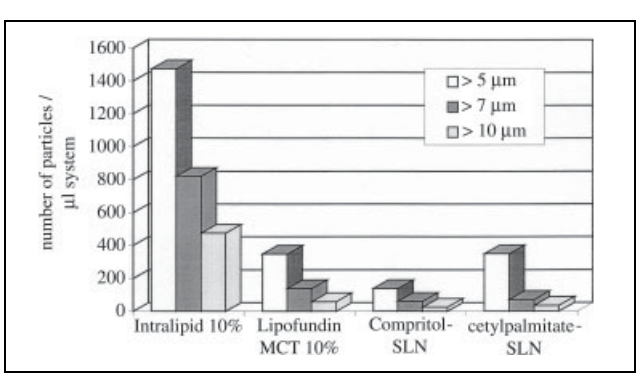


Fig. 1: Absolute number of particles having a diameter higher than 5, 7 and 10 μm/μl SLN formulation in comparison to parenteral fat emulsions

2.2. In vivo toxicity – histological results

Histological analysis of hepatic and splenic tissues of the cetyl palmitate group showed normal architecture (Fig. 2). Corresponding to the control, liver parenchyma proved to be a settled "lawn" of epithelia cells. No infiltrations or liver cell necrosis were found. The spleen showed a normal architecture of lymph follicles with a normal zone of marginal zone macrophages. For both liver and spleen, no signs of acute inflammation were found. To sum up, dissected organs of the cetyl palmitate group appeared to be inconspicuous.

Histological analysis of cuts obtained from the Compritol group (administration of 10% batch) showed pathological results (Fig. 3a). Hepatic tissue had infiltrations of mononuclear cells and showed Kupffer cell hyperplasia. In addition, liver cell necrosis (councilman bodies) has been observed. A more or less fatty degeneration of liver par-

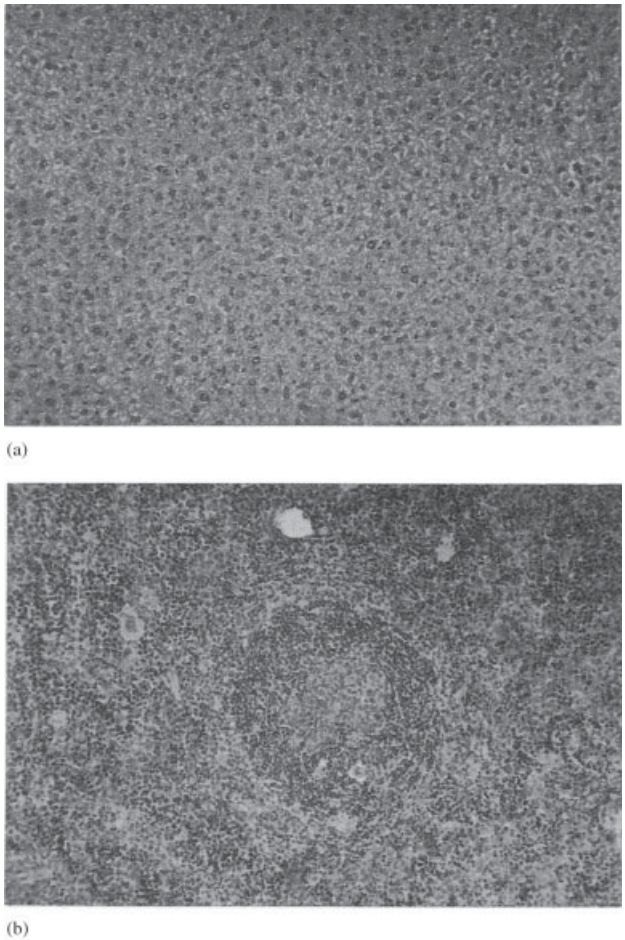
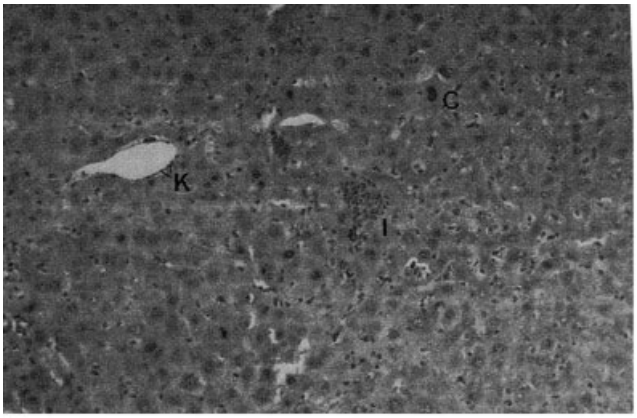
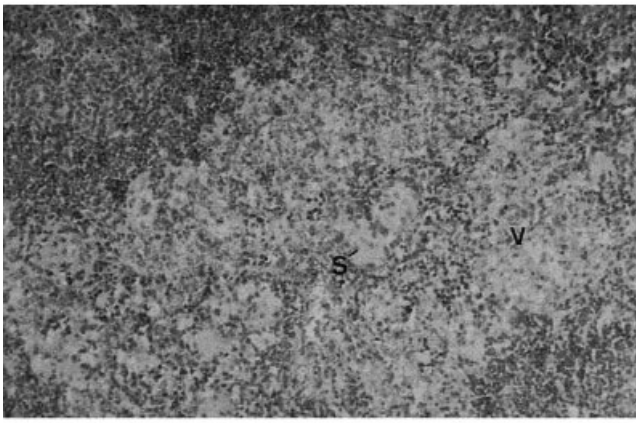


Fig. 2: Hepatic (a) and splenic (b) tissue histology after administration of the highest dose administration of the cetyl palmitate group

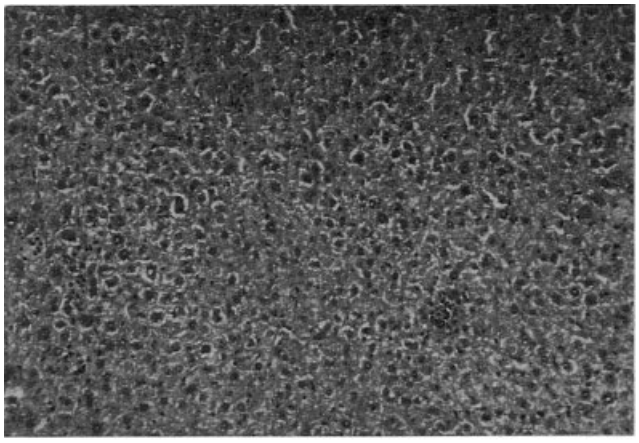


(a)

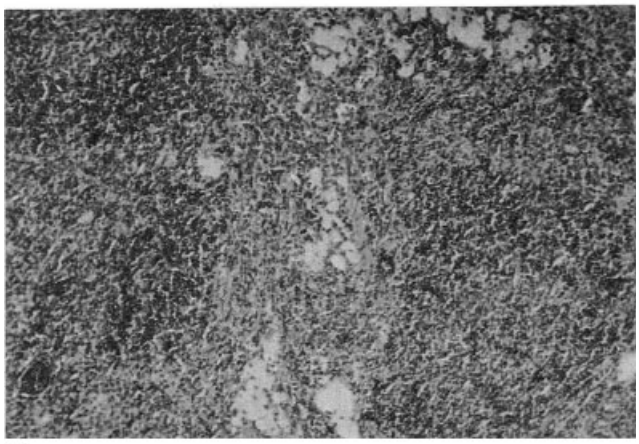


(b)

Fig. 3: Hepatic (a) and splenic (b) tissue histology after administration of the highest dose administration of the Compritol group. (K, Kupffer cells; I, infiltrations; C, councilman bodies; S, foam cells; V, fatty degeneration)



(a)



(b)

Fig. 4: Hepatic (a) and splenic (b) tissue histology six weeks after the last injection of the highest dose administration of the Compritol group

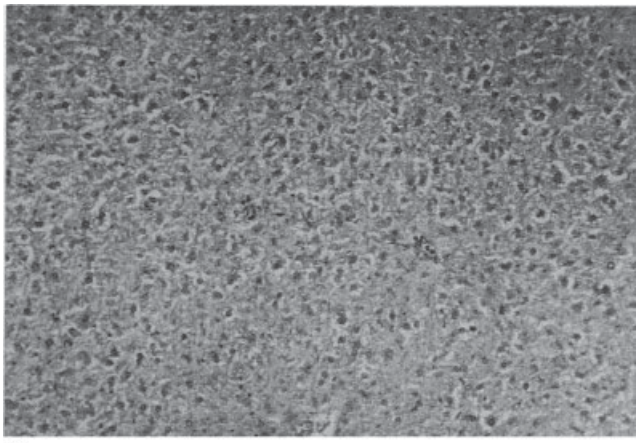
enchyma was clearly visible. Normal dehydrated liver tissue consisted of 2–5% fat stored in parenchyma cells. On top of those, structural fats like phospholipids and cholesterol are present. Only if this normal content of lipids is significantly exceeded, neutral fat becomes visible as drop-shaped storages (Gedigk and Brechtelsheimer 1986). Based on these pathological results, multiple administration of 10% Compritol SLN led to a fatty liver. Spleen showed a loss of normal architecture with destruction of lymph follicles (Fig. 3b). Zones of marginal zone macrophages were found to be reduced. Large lipid droplets within sinusoidal macrophages were observed in the area of the red pulp. The occurrence of foam cells – cells with honeycomb-like plasma spheres and fine dispersed lipids or lipoids – is also symptomatic for a manifest hyperlipidemia.

Mice of the Compritol group were kept for further six weeks after the last injection. Histological preparations were made and analysed (Fig. 4). Liver showed consolidation of mononuclear foci. Neither fresh infiltrations nor signs of acute inflammation were observed. A clear reduction in Kupffer cell hyperplasia was found. Obviously, architecture of liver-tissue was returning to normal state. Due to the ability of Kupffer cells to phagocytose councilman bodies and due to the ability of liver tissue to regenerate (Gedigk and Brechtelsheimer 1986), it can be assumed that the original lobular architecture is likely to be fully restored. The regeneration of lymphoid architecture and a normalisation of marginal zone macrophages in number were found in spleen, but a persistence of large lipid droplets in sinusoidal macrophages were still observed.

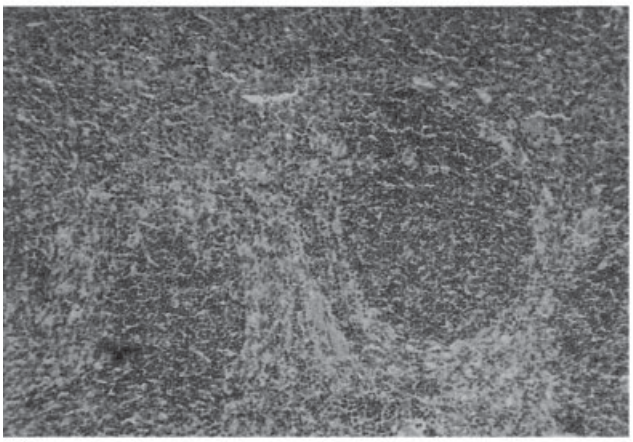
On the basis of the results obtained from the Compritol group a Compritol formulation with reduced lipid content (2.5% [m/m] instead of 10% [m/m]) was injected (Compritol LD batch). Simultaneously, the administered amount was reduced from 400 µl to 200 µl. Hepatic tissue of this so called Compritol LD group had only minimal mononuclear infiltrations (Fig. 5a). Kupffer cell hyperplasia was hardly recognizable. Parenchyma of the liver showed no signs of fatty degeneration. Splenic tissue had minimal disruption of splenic architecture and a slight reduction in marginal zone macrophages (Fig. 5b). There was no significant fatty degeneration in sinusoidal macrophages. The histological results showed only minimal changes.

2.3. In vitro degradation

High dosed Compritol formulations administered via the i.v. route resulted in an accumulation of lipid in liver and spleen. To explain this observation *in vitro* degradation studies have been performed. SLN were incubated in undiluted citrate stabilized human plasma. *In vitro* degradation of SLN was determined by monitoring the decrease of particle size (PCS diameter) versus time (Fig. 6). To interpret the PCS data, the background noise caused by the light scattering of the plasma has to be taken into account (measured virtual size for plasma: 97 nm). Due to



(a)



(b)

Fig. 5: Hepatic (a) and splenic (b) tissue histology after administration of the lowest dose administration of the Compritol group

the interference of SLN particles with the plasma signal, only a relative degradation of SLN approaching the virtual size can be monitored. Nevertheless, a clear statement is possible. Particle size of cetyl palmitate SLN only decreased from 269 nm to 149 nm within the first two hours, while particle size of the Compritol SLN decreased from 242 nm to 214 nm. Within the first two hours the decrease in size is 44.6% for cetyl palmitate SLN and 11.6% for Compritol SLN. After 24 h of incubation of SLN batches with human plasma, particle size of cetyl palmitate SLN had decreased to the virtual background size for plasma (97 nm). These results prove that *in vitro* degradation velocities of the injected lipids differ.

3. Discussion

Based on the results from particle size and particle size distribution measurements, the produced SLN batches can be considered as suitable for intravenous administration. With regard to contamination by microparticles, the SLN batches proved to be of similar quality as parenteral fat emulsions available on the pharmaceutical market (Intralipid^{TM}, Lipofundin^{TM}). These assessments were confirmed by the *in vivo* data. No signs of acute toxicity (e.g. emboli) were observed after i.v. injection. The animals behaved inconspicuous.

Data obtained from histological examinations have to be discussed with regard to the physiological metabolism of lipids. Long chain fatty acids are resembled to triacylglycerols in enterocytes of the duodenum and jejunum. Chylomicrons, water dispersible vesicles (ca. 1 µm in size), are composed of 84% triacylglycerols, 7% cholesterine, 7% phospholipids and 2% proteins. Via the lymphatic system chylomicrons finally reach the bloodstream. Within the bloodstream parts of the triacylglycerols are degraded by lipoprotein lipases. Then, the fatty acids are either stored in fatty tissue or are converted into energy within muscle tissue. The chylomicron remnants (including triacylglycerols) reach the liver. If an excessive supply of triacylglycerols is present, the liver cannot handle this situation even though the secretion of VLDL is increased. In this case, triacylglycerols are stored within hepatocytes. The triacylglycerols become visible within the cytoplasma as large lipid droplets. In histological cuts of the Compritol group (high dosed administration of 10% SLN) lipid droplets were found both in hepatic and splenic tissues. It is reported that in the liver these lipid droplets can lead to a functional disturbance (Gedigk and Tolovic 1986). Pathological storages will lead to cell necrosis. This results in councilman bodies as observed in histological cuts of hepatic tissue of the Compritol group. Normally, the storage of lipids within hepathocytes is reversible. Fatty degeneration of liver turns back to normal if the supply of fats is reduced. This statement could be clearly confirmed for the Compritol group. Six weeks after the last administration of Compritol SLN, liver architecture is nearly recovered. Due to the fact that Kupffer cells are able to phagocytose councilman bodies, it can be assumed that the original lobular architecture is going to be restored.

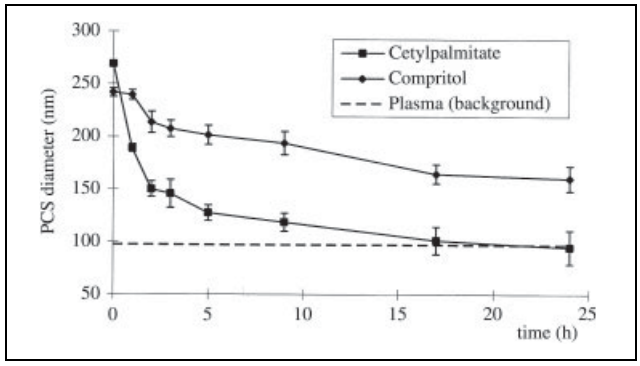


Fig. 6: *In vitro* degradation of SLN incubated in human plasma

The mobilisation of fat within fatty tissue or liver and therefore also the mobilisation of restored fat in hepathocytes is limited by a highly specific triacylglycerol lipase (Karlson 1984). This enzyme is controlled by several hormones such as adrenaline or glucagon. The accumulation of Compritol might be therefore attributed to a lack of up regulation of this triacylglycerol lipase. Mono- and diacylglycerols, and probably also waxes such as cetyl palmitate, are degraded by more unspecific lipases and stearases. This fact might explain the lack of cetyl palmitate accumulation. Differences in degradation velocity of Compritol and cetyl palmitate were confirmed by *in vivo* degradation studies in human plasma. The lipases and estearases present in the plasma were able to degrade the cetyl palmitate SLN relatively fast.

Kupffer cell hyperplasia of the liver and the fatty degeneration of the red pulp enriched with macrophages were observed in the Compritol group. This can also be explained with regard to metabolism of lipids. Phagocytosis of fat is reported by Gedigk and Totovic (1986) if the content of lipids is enriched. Uptake of highly dosed SLN by macrophages is more likely in this case. After ingestion of SLN, the degradation of the lipid matrix takes

place within the lysosomes. Here again, a lack of up regulation of the specific triacylglycerol lipase might explain an accumulation of Compritol instead of cetyl palmitate. Low dosed Compritol SLN (= Compritol LD group) are well tolerated. As a result, the use of Compritol is limited by side effects when administered in extremely high doses. In this study, the administered dose of Compritol corresponds to 100 g of lipid given six-fold to men (75 kg) in a bolus injection. One has to stress that the administered dose of lipid would be distinctly lower in therapy with SLN as drug carrier (e.g. single dose of 1 g lipid, corresponding to 10 ml of 10% SLN dispersion).

In conclusion, the studies showed that SLN composed of cetyl palmitate are suitable for i.v. administration even though cetyl palmitate as a wax is not a physiological compound. Even a relatively high dose was well tolerated after multiple injections. The use of Compritol is limited by side effects when administered in extremely high doses (e.g. Compritol: 100 g of lipid given six-fold to men). These side effects were attributed to the slow degradation of the Compritol matrix as shown by performing *in vitro* studies in human plasma. However, Compritol SLN are considered as being suitable for i.v. use because the administration volume will be much smaller in therapy. This first *in vivo* toxicity study of SLN indicates a good tolerability of the carrier by the body, which is an important pre-requisite for the potential use in clinical practice.

4. Experimental

4.1. Lipids and emulsifiers

Compritol ATO 888 (glycerol behenate), a lipid with GRAS status (generally recognized as safe [FDA]), was a gift from Gattefossé (Weil, Germany). Cetyl palmitate was purchased from Caelo (Hilden, Germany). Polysorbate 80 (Tween 80) was a gift from Thomae (Biberach/Riss, Germany). Lipoid S75 (soy lecithin) was obtained from Lipoid KG (Ludwigshafen, Germany). All materials were used as received.

4.2. Animals

In vivo experiments were performed using male C57 BL/6 mice (own breeding). The animals were kept under constant conditions (temperature, humidity) in a 12 h/12 h light/dark cycle. Animals were fed with a standard diet and water *ad libidum*.

4.3. Production of SLN batches

SLN batches (lipid content 10% [m/m], emulsifiers: 3.4% [m/m] Tween 80 and 0.6% [m/m] Lipoid S75) produced on lab scale were prepared according to Weyhers (1995), adding an aqueous phase containing the emulsifier heated at 80 °C to the melted lipid. The mixture was stirred using an Ultra Turrax T25 Janke and Kunkel GmbH & Co KG, Staufen, Germany) for 1 min at 10 000 rpm. The obtained pre-emulsion was homogenized using a Labsonic 2000 ultrasonic probe (Braun Melsungen AG, Melsungen, Germany). The power output for 2 min was 200 or 300 W, respectively. Additionally, a low dose Compritol containing SLN batch (Compriton LD batch) was prepared by dilution of the Compritol batch. Thus, the lipid content was reduced from 10% [m/m] to 2.5% [m/m]. Finally, isotonization of all batches was adjusted by adding glucose.

4.4. Physicochemical characterization of SLN

Mean particle size, polydispersity index (PI) and volume distribution were determined by photon correlation spectroscopy (PCS) using the Malvern Zetasizer IV (Malvern Instruments, UK), and by laser diffractometry (LD) using the Coulter LS 230 (Coulter Electronics, Krefeld, Germany) yielding the volume distribution of the particles. For the determination of particle aggregates the diameters D90%, D95% and D99% of the laser diffraction particle size analysis were used. The D99% value indicates that 99% of the detected particles (volume distribution) are smaller than the listed values. Large particles (microparticles) limiting the i.v. administration were quantified using a Coulter Counter Multisizer II (Coulter Electronics, Krefeld, Germany). To assess the suitability of SLN batches for i.v. injection, their content of microparticles was compared with fat emulsions for parenteral nutrition commercially available on the pharmaceutical market, i.e. Lipofundin™ (B. Braun Melsungen AG, Germany) and Intralipid™ (Pharmacia, Stockholm, Sweden).

4.5. Intravenous administration of SLN batches

Mice (6 animals per group) were given 400 µl of SLN dispersion (lipid content 10% [m/m]) by i.v. administration via the tail vein. Administration was repeated for six times within a period of 20 days. Additionally, a low dose Compritol containing SLN formulation was administered (named "Compritol LD batch", LD = low dose). In this case, the administered volume was 200 µl of SLN dispersion (lipid content 2.5% [m/m]).

4.6. Determination of the in vivo toxicity

Four days after the administration of SLN, mice were sacrificed by cervical dislocation according to the EG guideline 86/609/EEC. Liver and spleen (main organs of the MPS) were dissected. Organs were taken for histological analysis. Samples were fixed, dehydrated through graded ethanol and embedded in wax. Microtom sections were cut at 3 µm using a Microm HM 400 (Fa. Heidelberg, Germany) and stained with haematoxylin eosin.

4.7. Determination of in vitro degradation

A volume of 10 µl of each SLN dispersion (10% lipid content [m/m]) was incubated in 2 ml undiluted citrate stabilised, non-inactivated human plasma at 37 °C. The degradation of SLN was determined by monitoring the decrease in particle size (mean diameter) versus the time using photon correlation spectroscopy. This technique was used as described previously for the degradation of alkylcyanoacrylate nanoparticles (Müller 1992) and also for SLN (Müller 1995).

References

Bock T (1994) Emulsionen als parenterale Arzneistoffträgersysteme, Herstellung, Charakterisierung und Optimierung. PhD Thesis. Kiel, Christian Albrechts University Kiel.

Freitas C, Lucks JS, Müller RH (1994) Effect of storage conditions on long-term stability of solid lipid nanoparticles (SLN) in aqueous dispersion. Eur J Pharm Sci 2: 178.

Gedigk P, Bechtelsheimer H (1986) Große Verdauungsdrüsen: Leber. in: Eder M, Gedigk P (eds.) Lehrbuch der allgemeinen Pathologie und der Pathologischen Anatomie, 32. Auflage, Berlin, Heidelberg, New York, London, Paris, Tokyo, Springer Verlag: 571–598.

Gedigk P, Tolovic V (1986) Zell und Gewebsschäden, in: Eder M, Gedigk P (eds) Lehrbuch der allgemeinen Pathologie und der Pathologischen Anatomie, 32. Auflage. Berlin, Heidelberg, New York, London, Paris, Tokyo, Springer Verlag: 32–44.

Jenning V, Schäfer-Korting M, Gohla S (2000) Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties. J Control Release 66: 115–126.

Karlson P (1984) Kurzes Lehrbuch der Biochemie. Stuttgart, New York, Georg Thieme Verlag.

Mehnert W, Mäder K (2001) Solid lipid nanoparticles – Production, characterization and applications. Adv Drug Deliv Rev 47: 165–196.

Müller RH, Dingler A, Runge SA, Schneppe T, Gohla S (2000a) Large scale production of solid lipid nanoparticles (SLN™) and nanosuspensions (DissoCubes™), in: Wise D (ed.) Handbook of Pharmaceutical Controlled Release Technology. New York, Marcel Dekker Inc.: 359–376.

Müller RH, Heinemann S (1992) Fat emulsions for parenteral nutrition. I. Evaluation of microscopic and laser light scattering methods for the determination of the physical stability. Clinical Nutrition 11: 223–236.

Müller RH, Lehrm C, Herbort J, Blunk T, Couvreur P (1992) Alkylcyanoacrylate drug carriers: I Physicochemical characterization of nanoparticles with different alkyl chain length. Int J Pharm 84: 1–11.

Müller RH, Lippacher A, Gohla S (2000b) Solid Lipid Nanoparticles (SLN) as Carrier System for the Controlled Release of Drugs, in: Wise D (ed.) Handbook of Pharmaceutical Controlled Release Technology, 377–391.

Müller RH, Lucks JS (1991) Arzneistoffträger aus festen Lipidteilchen, Feste Lipidnanosphären (SLN). EP 0605497.

Müller RH, Maaßen S, Weyhers H, Mehnert W (1996a) Phagocytic uptake and cytotoxicity of solid lipid nanoparticles (SLN) sterically stabilized with poloxamine 908 and poloxamer 407. J Drug Target 4: 161–170.

Müller RH, Maaßen S, Weyhers H, Specht F, Lucks JS (1996b) Cytotoxicity of magnetite-loaded polylactide, polylacttide/glycolide particles and solid lipid nanoparticles. Int J Pharm 138: 85–94.

Müller RH, Mäder K, Gohla S (2000c) Solid lipid nanoparticles (SLN) for controlled drug delivery – A review of the state of art. Eur J Pharm Biopharm 50: 161–177.

Müller RH, Mehnert W, Lucks J-S, Schwarz C, zur Mühlen A, Weyhers H, Freitas C, Rühl D (1995) Solid lipid nanoparticles (SLN) – An alternative colloidal carrier system for controlled drug delivery. Eur J Pharm Biopharm 41: 62–69.

Müller RH, Schwarz C, zur Mühlen A, Mehnert W (1994). Incorportion of lipophilic drugs and drug release profiles of solid lipid nanoparticles. Proc Int Symp Control Rel Bioact Mater 21.

Müller RH, Souto EB (2005) Lipid nanoparticles (SLN and NLC) for drug delivery, in: Kumar R, Tabata Y, Domb A: Nanoparticles for Pharmaceutical Applications. American Scientific Publishers.
Schwarz C, Mehnert W (1997) Freeze-drying of drug-free and drug-loaded solid lipid nanoparticles (SLN). Int J Pharm 157: 171–179.
Schwarz C, Mehnert W, Lucks JS, Müller RH (1994a) Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. J Control Release 30: 83–96.
Schwarz C, Mehnert W, Müller RH (1994b) Lyophilisation of solid lipid nanoparticles (SLN). Eur J Pharm Sci 2: 177.
Souto EB, Wissing SA, Barbosa CM, Müller RH (2004) Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. Int J Pharm 278: 71–77.
Weyhers H (1995) Feste Lipid Nanopartikel (SLN) für die gewebsspezifische Arzneistoffapplikation, Herstellung, Charakterisierung oberflächenmodifizierter Formulierungen. PhD Thesis. Berlin, Freie Universität Berlin.