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Surfactant, but not the size of solid lipid nanoparticles (SLN) influences viability and cytokine production of macrophages

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Abstract

After intravenous (i.v.) injection, solid lipid nanoparticles (SLN) interact with mononuclear cells. Murine peritoneal macrophages were incubated with SLN formulations consisting of Dynasan 114 coated with different surfactants. The present study was performed to examine the impact of surfactants, which are important surface defining components of SLN, on viability and cytokine production by macrophages. Cytotoxicity, as assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) test, was strongly influenced by the surfactant used being marked with cetylpyridinium chloride- (CPC-) coated SLN at a concentration of 0.001% and further increased at SLN concentrations of 0.01 and 0.1%. All other SLN formulations — containing Poloxamine 908 (P908), Poloxamer 407 (P407), Poloxamer 188 (P188), Solutol HS15 (HS15), Tween 80 (T80), Lipoid S75 (S75), sodium cholate (SC), or sodium dodecylsulfate (SDS) — when used at the same concentrations reduced cell viability only slightly. None of the SLN formulations tested induced cytokine production but a concentration-dependent decrease of IL-6 production was observed, which appeared to be associated with cytotoxic effects. IL-12 and TNF-α were detected neither in supernatants of macrophages treated with SLN at any concentration nor in those of untreated cells. In contrast to the type of surfactant, the size of SLN was found neither to affect cytotoxicity of SLN nor to result in induction or digression of cytokine production by macrophages. In conclusion, testing the effects of surfactants on SLN on activity of macrophages is a prerequisite prior to in vivo use of SLN.

Keywords: Solid lipid nanoparticles; Surfactant; Surface modification; Size; Peritoneal macrophages; Cytokines; Cytotoxicity

1. Introduction

Surface properties of solid lipid nanoparticles (SLN) critically determine their fate after intra-

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venous (i.v.) injection (Müller et al., 1995). If taken up by mononuclear phagocytes (MP), a significant amount of SLN will be trapped in organs of the mononuclear phagocytic system (MPS). In most therapeutic applications, this uptake needs to be avoided in order to secure a sufficient amount of drug-containing SLN in the blood stream to be available for targeting to sites other than the MPS. Examples are targeting of carriers to the brain, e.g. treatment of toxoplasmosis (Schöler et al., 2001), or to the bone marrow, e.g. delivery of growth inhibitors and growth factors in cancer therapy. Further applications are the passive targeting to tumours across the fenestrated endothelium of the blood capillaries, requiring non-recognised, long-circulating carriers in the blood. Several surfactants have been identified that lead to a decreased uptake of particulate delivery systems by MP (Illum et al., 1987; Rudt and Müller, 1992, 1993b; Rudt et al., 1993; Rühl et al., 1995; Müller and Olbrich, 1999). In contrast, an increased uptake by phagocytic cells is needed when SLN are used as delivery systems for antibiotics against intracellular microorganisms or as adjuvants in combination with specific antigens for immunisation.

In order to target SLN to specific tissue sites, their surface needs to be modified (Müller et al., 1995). Using specific surface modifications, the passage of SLN through barriers — such as the blood–brain barrier (BBB) — appears feasible. Polymer particles coated with polysorbate 80 (Tween 80) are able to deliver drugs through the BBB (Alyautdin et al., 1995, 1997, 1998). SLN loaded with about 30% diminazene could be successfully located at the BBB (Geßner et al., 2001).

In order to develop SLN for i.v. use, detailed information about the interaction of SLN with phagocytic cells — such as MPs and granulocytes — which are the first cells to interact with SLN in the blood stream, is a prerequisite. Interaction of phagocytic cells with foreign bodies — such as drug delivery systems — may result in phagocytic uptake and uncontrolled release of pro-inflammatory cytokines such as interleukin-(IL-) 1, IL-6, IL-12, and tumor-necrosis-factor (TNF)-α (Van Furth et al., 1972; Baumann and Gauldie, 1994) and/or cell death.

Interactions of mononuclear cells with particulate systems were found to be influenced by their composition, size and concentration (Maaßen, 1994; Müller et al., 1997a,b). In this regard, the lipid matrix, e.g. consisting of stearic acid and dimethyl-dioctadecylammonium, but not triglycerides, cetyl palmitate or paraffin, influenced viability and cytokine production by murine peritoneal macrophages (MΦ), one class of MNs (Schöler et al., submitted). The size of SLN ranging from 0.2 to 7 μm did not determine viability or cytokine production (Schöler et al., submitted).

In addition to the lipid matrix, surface properties, e.g. hydrophobicity and surface charge, have a major impact on phagocytic uptake of particulate drug delivery systems (Van Oss et al., 1975, 1983). The surfactant also influences the amount and the velocity of phagocytosis of SLN by human granulocytes (Müller et al., 1997a). Sterically stabilising surfactants such as block-copolymers, especially Poloxamer 407 and Poloxamine 908, led to a reduction of phagocytosis of SLN by freshly isolated human granulocytes (Müller et al., 1997a) and by HL 60 cells (Müller et al., 1997b,c). In addition, MΦ failed to recognise polystyrene model drug carriers coated with Poloxamer 407 or Poloxamine 908 (Illum et al., 1986, 1987). Intensive in vitro cell culture studies have been performed to study the particle–cell interaction and the resulting particle uptake as a function of particle size and simultaneously surface modification by adsorption of a full range of surface modifiers, (Rudt and Müller, 1992, 1993a,b,c; Rudt et al., 1993).

Since the surfactant of SLN affects surface properties of SLN, we investigated whether different surfactants on SLN, including charged, non-charged and/or sterically stabilising surfactants, may determine cytotoxicity of SLN and/or modulate the cytokine production by peritoneal MΦ. Furthermore, experiments were performed to investigate further the impact of SLN size on their interaction with MΦ as a function of the stabilising surfactant.

2. Materials and methods

2.1. Materials

Dynasan 114 was kindly provided by Condea (Witten, Germany). Poloxamer 188 (P188), Poloxamer 407 (P407), and Solutol HS15 (HS15) were purchased from BASF (Ludwigshafen, Germany), Poloxamine 908 (P908) from CH Erbslöh (Düsseldorf, Germany), SDS from Bio-Rad Laboratories (Munich, Germany) and Tween 80 (T80) from ICI Surfactants (Eversberg, Belgium). Lipoid S75 (lecithin) (S75) was obtained from Lipoid KG (Ludwigshafen, Germany). Sodium cholate (SC), cetylpyridinium chloride (CPC), medium-chained triglycerides, and soybean oil as well as 3,3′, 5,5′-tetramethylbenzidine tablets (TMB) and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (Deisenhofen, Germany). Glycerol for isotonisation was purchased from Merck (Darmstadt, Germany). Lipofundin MCT 10% for control was purchased from Braun Melsungen (Melsungen, Germany). Polystyrene beads of different sizes (small: 250 nm, medium: 512 nm, large: 3.25 μm) were purchased from Polyscience (St. Goar, Germany). Giemsa and May-Grünwald solutions used for staining were obtained from Merck (Darmstadt, Germany). Brewer's thioglycollate was from Difco Laboratories (Detroit, MI, USA). Cell culture reagents were obtained from Biochrom (Berlin, Germany). Pansorbin was obtained from Calbiochem (Bad Soden, Germany). All reagents used for enzyme-linked immunosorbent assay (ELISA) were obtained from Pharmingen (San Diego, CA, USA), except streptavidine horseradish peroxidase (Amersham, Little Chalfont, Great Britain).

2.2. SLN preparation and size measurement

SLN were produced by high-pressure homogenisation as described previously (Müller et al., 1995; Schöler et al., 2000a,b). Briefly, the melted lipid (10% w/w) was added to a hot aqueous surfactant solution (1% w/w). This mixture formed a pre-emulsion after high-speed stirring for 1 min with an Ultra turrax T 25 (Janke and Kunkel, Staufen, Germany). SLN were obtained by high-pressure homogenisation using a Micron LAB 40 homogeniser (APV-Gaulin, Lübeck, Germany). Different particle sizes were obtained by changing the production parameters pressure and cycle number. Particles were produced under aseptic conditions using a laminar airflow chamber.

Particle size and width of distribution (polydispersity index) were determined by photon correlation spectroscopy (PCS) (Malvern Zetasizer IV, Malvern Instruments, Herrenberg, Germany) or laser diffractometry (Coulter LS 230, Coulter

Table 1
Diameters of SLN consisting of Dynasan 114 coated with different surfactants as measured by photocorrelation spectroscopy ($n = 3$) (concentration of SLN dispersion: 10.0% (w/w) lipid 1.0% (w/w) surfactant)

Surfactant	Abbreviation of SLN	Diameter (nm) ± standard deviation	Polydispersity index ± standard deviation
Poloxamine 908	P908	253 ± 1	0.14 ± 0.03
Poloxamer 407	P407	252 ± 4	0.21 ± 0.02
Poloxamer 188	P188	181 ± 1	0.18 ± 0.01
Solutol HS15	HS15	176 ± 1	0.13 ± 0.01
Tween 80	T80	146 ± 1	0.21 ± 0.01
Lipoid S75	S75	228 ± 1	0.11 ± 0.03
Sodium cholate	SC	254 ± 2	0.19 ± 0.03
Lipoid S75/sodium cholate	S75/SC	192 ± 2	0.12 ± 0.03
Sodium dodecyl-sulfate	SDS	167 ± 3	0.19 ± 0.01
Cetylpyridinium chloride	CPC	160 ± 1	0.19 ± 0.01

Electronics, Miami, FL, USA) (shown in Tables 1 and 3).

2.3. Murine peritoneal macrophages

Murine peritoneal cells were collected from peritoneal cavities of locally bred female BALB/c mice after intraperitoneal injection of 0.5 ml of 3% Brewer's thioglycollate according to a procedure described previously (Schöler et al., 2000a,b). In brief, donor mice were killed in a CO_2-atmosphere and cells harvested by lavaging the peritoneal cavity. Harvested cells were pooled and counted using a hemocytometer. Viability was determined by trypan blue exclusion. Differential counts were performed on fixed Pappenheim smears. Enumeration of harvested cells revealed $77.3 \pm 9.1\%$ mononuclear cells and $22.1 \pm 8.5\%$ lymphocytes. Adherent cells (MΦ) were suspended at 1×10^6 per ml in RPMI 1640 in the presence of 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin, and seeded into 96-well flat bottom plates. MΦ were incubated in the presence of SLN suspensions, 75.0 μg/ml Pansorbin (Protein A of *Staphylococcus aureus*), polystyrene latex bead suspensions (PSB), or Lipofundin MCT 10% (LIP) emulsions at different concentrations dispersed in cell medium. After 1 h of incubation extracellular particles were rinsed off. For cytokine detection, supernatants were harvested at 20 h of incubation and stored at −70°C until testing. Experiments were performed in triplicate and repeated at least twice.

2.4. Cytokine assay

The concentrations of cytokines (IL-6, IL-12, TNF-α) in supernatants were determined by two-site ELISAs in Nunc-Immuno modules according to standard procedures. Briefly, purified anti-cytokine antibodies were used as primary antibodies, biotinylated rat anti-mouse cytokines as the secondary antibodies, and streptavidine-conjugated peroxidase as the developing reagent with 3,3′,5,5′-TMB tablets as substrate (Schöler et al., 2000a,b). Cytokine concentrations were determined by reference to standard curves constructed with fixed amounts of mouse recombinant IL-6, IL-12 or TNF-α. The optical density (OD) was measured using an automated ELISA plate reader at 405 nm with a 620-nm reference filter. Data came from triplicate determinations and were expressed in nanograms per millilitre (mean ± standard deviation). The sensitivity limits for the different assays were as follows: IL-6, 0.20 ng/ml; IL-12, 0.15 ng/ml; TNF-α, 0.15 ng/ml.

2.5. Nitric oxide (NO) determination

NO determination was performed using the Griess reaction; details of the method can be found in Nithipatikom et al. (1996).

2.6. Viability

Viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay (Mosmann, 1983). Cells were incubated in parallel with cells used for cytokine detection by ELISA. Four h prior to the 20-h time point, 50 μl MTT solution (Sigma) (2.5 mg/ml MTT in PBS) was added and incubated for 4 h at 37°C in a 5% CO_2-incubator. Surfactants were removed and the blue salt formed by living cells dissolved in a 20% sodium dodecylsulfate solution (SDS). Absorbance was determined at 550 nm using an automated ELISA plate reader. Viability was expressed in percent compared to untreated cells. Experiments were performed in triplicate and repeated at least twice.

2.7. Statistical analysis

Statistical analysis was performed using the unpaired, two-tailed Student's *t*-test.

3. Results and discussion

(Schöler et al., submitted) The nature of the lipid matrix and the concentration, but not the size of SLN, dramatically determine cytotoxicity of SLN on MΦ, (Schöler et al., Submitted) We therefore performed the present study to examine the influence of the second component of SLN,

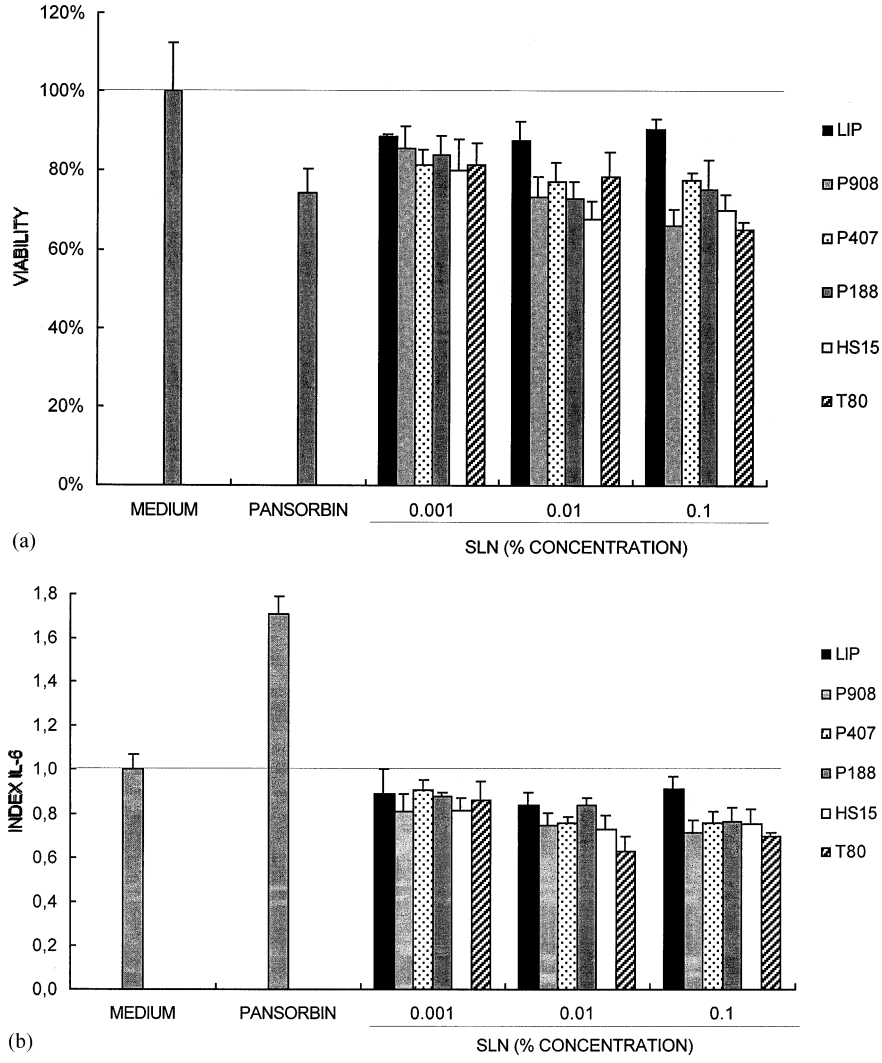

Fig. 1. (a) Viability of peritoneal macrophages after 20 h of in vitro culture with different concentrations of SLN consisting of Dynasan 114 coated with different surfactants and with medium, Pansorbin, or Lipofundin MCT (LIP) ($n = 3$, $\pm$ standard deviation). Viability of macrophages cultured in medium was considered 100%. (b) Secretion of IL-6 by ELISA in supernatants of the same cells after 20 h of in vitro culture with the same ingredients as tested in (a). IL-6 levels are shown as mean index IL-6 $\pm$ standard deviation ($n = 3$).

the surfactant, on their interaction with MΦ. Furthermore, experiments were performed to investigate more intensively the effect of the SLN particle size on viability and cytokine production of MΦ, i.e. effect of size using different surfactants.

In a first series of experiments, SLN coated with uncharged, sterically stabilising surfactants was tested. No major cytotoxic effects were found following incubation of MΦ with SLN coated with P908, P407, P188, HS15 and T80 at concentrations of 0.001% or lower (Fig. 1a). A slight reduction of cell viabilities to $67.7 \pm 4.6\%$ was detected when concentrations of HS15 SLN were increased to 0.01%. At concentrations of 0.1%, P908 and T80 SLN resulted in slight decrease of viability ($66.1 \pm 4.3\%$ and $65.0 \pm 2.1\%$, respectively). Compared to LIP — used as non-toxic

control, since it is commonly used for parenteral fat nutrition — a slight concentration-dependent cytotoxicity was exerted by all SLN formulations at concentrations of between 0.001 and 0.1% (Fig. 1a). In addition to cytotoxic effects, we examined the production of IL-6, IL-12 and TNF-α. IL-6 production by MΦ following incubation with all of the above-mentioned SLN formulations was reduced at concentrations of 0.001–0.1% in a concentration-dependent manner (Fig. 1b). The decrease of cytokine secretion paralleled cytotoxic effects of these SLN. Therefore, the IL-6-depressing effects of SLN are most likely attributable to their cytotoxic effects.

TNF-α and IL-12 were detectable neither in supernatants of untreated cells nor after incubation with any of the above-mentioned SLN formulations or control emulsion (data not shown). Stimulation of these cells with Pansorbin — performed to examine the capacity of cells to up-regulate cytokine production — resulted in significantly higher IL-6 concentrations (Fig. 1b), as well as detection of TNF-α (1.82 ± 0.76 ng/ml) and IL-12 (2.32 ± 0.97 ng/ml). Thus, in our assay system cultivated MΦ retained their potential to up-regulate cytokine production. Incubation of MΦ with P908, P407, P188, HS15 and T80 SLN did not up-regulate pro-inflammatory cytokines but rather resulted in down-regulation of cytokine production due to cytotoxicity of these SLN formulations.

Treatment of MΦ with another set of SLN coated with charged surfactants again reduced viability of MΦ in a concentration-dependent manner (Fig. 2a). Remarkable cytotoxic effects were exerted by the positively charged CPC SLN at 0.01 and 0.1% (Fig. 2a). It should be noted that in equal concentrations (CPC concentration present in 0.1% aqueous SLN dispersion), the cytotoxicity of CPC was increased when incorporated in SLN (Fig. 2a) compared to the equally concentrated solution of the surfactanat alone (Table 2, last line). These findings are in good agreement with the paper by Olivier et al. (1999), reporting an increased cytotoxicity of Tween 80 in combination with nanoparticles; obviously, it is very important in which conformation the surfactant is adsorbed on the nanoparticle surface, i.e. shielding or exposing the functional group responsible for the cytotoxic effect. Incubation of MΦ with SLN coated with Lipoid S75 (S75) at a concentration of 0.1% resulted in a cell viability of $73.6 \pm 5.1\%$. Viabilities of cells incubated with SLN coated with SC or with a mixture of Lipoid S75 and SC (S75/SC) at concentrations of 0.1% did not differ significantly from each other ($62.0 \pm 3.8\%$ and $67.5 \pm 2.7\%$, respectively). At SLN concentrations below 0.001%, viability of cells did not differ from that observed with SLN at a concentration of 0.001% (data not shown). In parallel with these findings, IL-6 production, too, was reduced in a concentration-dependent manner following incubation with the same set of SLN (Fig. 2b). Therefore, the surfactant — like the lipid matrix as has been shown by us (Schöler et al., submitted) — influences strongly the interaction of SLN with MΦ.

Surfactants used as components of SLN, when tested alone at equivalent concentrations to those used in SLN, did not result in reduced viabilities of MΦ (Table 2). Furthermore, none of the surfactant solutions led to a marked depression of IL-6 levels (Table 2). Therefore, cytotoxic effects, induced by SLN formulations containing specific surfactants, did not appear to accrue solely from the surfactants used.

Cytotoxicity of drug carrier systems may be caused by various mechanisms. Cytotoxic effects of polycyanoacrylate nanoparticles have been reported to be due to their adherence to the cell membrane and subsequent degradation of particles with the release of toxic degradation products (Lherm et al., 1992; Maaßen et al., 1993a). Extracellular toxicity caused by adherence of cells was reported not to be a likely mechanism of cytotoxicity (Müller et al., 1997a). The cytotoxicity of CPC SLN as observed in our experiments is remarkable, since CPC is commonly used as a cationic antiseptic for mouth rinsing at concentrations of 0.05%. Its antimicrobial activity is based on its interference with bacterial membrane functions (Roberts and Addy, 1981). Cytotoxic effects on MΦ could therefore be explained by adherence to the cell membrane and interaction of the positively charged CPC with the negatively charged cell membrane. At first glance, it appears surpris-

ing that no cytotoxic effects were observed when MΦs were incubated with CPC solutions at concentrations equivalent to those used in CPC SLN (resulting in effective CPC concentrations of 0.00001–0.001%). The CPC molecules in the reference solution are evenly distributed in the cell culture medium and thus are adsorbed evenly on the cell surface. In contrast, when CPC-stabilised SLN adhere to the cell surface, there is a high concentration of CPC at these adsorption points, which may cause membrane damage that results in the observed cytotoxicity. Similar effects were reported for cytotoxic degradation products of polycyanoacrylate (PCA) nanoparticles (Lherm et al., 1992). (Lherm et al.) reported that PCA nanoparticles adhered to the cell membrane (similar to the cationic SLN adhering to the negatively charged cell membrane), leading to degradation of

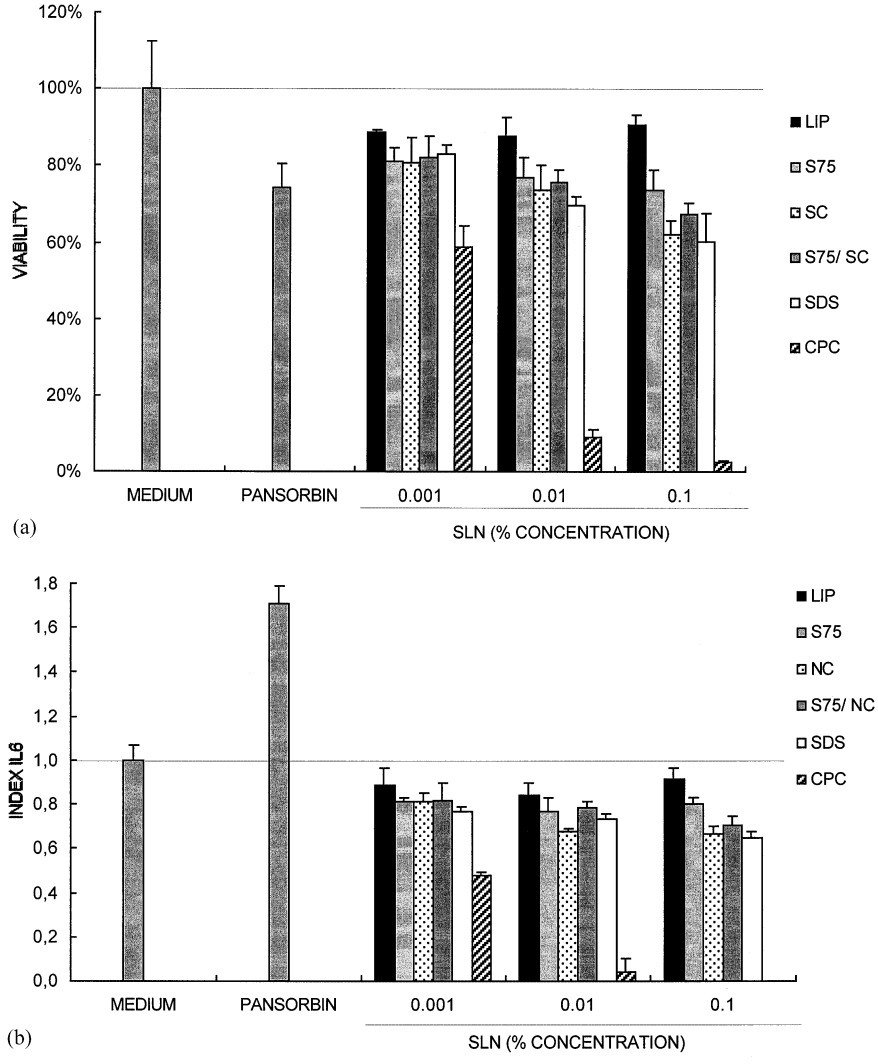

Fig. 2. (a) Viability of peritoneal macrophages after 20 h of in vitro culture with different concentrations of SLN consisting of Dynasan 114 coated with different surfactants and with medium, Pansorbin, or Lipofundin MCT (LIP) ($n = 3$, $\pm$ standard deviation). Viability of macrophages cultured in medium was considered 100%. (b) Secretion of IL-6 by ELISA in supernatants of the same cells after 20 h of in vitro culture with the same ingredients as tested in (a). IL-6 levels are shown as mean index IL-6 $\pm$ standard deviation ($n = 3$).

Table 2
Viability and IL-6-production of peritoneal macrophages after 20 h of in vitro culture with surfactant solutions at a concentration of 0.01% (equivalent to a SLN concentration of 0.1%) and with medium ($n = 3$, $\pm$ standard deviation)

Surfactant	Viability (%)[a] $\pm$ standard deviation	Index IL-6[b] $\pm$ standard deviation
Medium	100.0 ± 12.6	1.00 ± 0.07
Poloxamine 908	90.2 ± 3.0	0.86 ± 0.08
Poloxamer 407	90.4 ± 7.6	0.90 ± 0.01
Poloxamer 188	87.3 ± 0.9	0.91 ± 0.06
Solutol HS15	93.3 ± 5.3	0.90 ± 0.06
Tween 80	90.9 ± 5.5	0.85 ± 0.04
Lipoid S75	86.0 ± 3.3	0.86 ± 0.04
Sodium cholate	92.8 ± 4.9	0.93 ± 0.10
Lipoid S75/Sodium cholate	89.2 ± 1.5	0.89 ± 0.10
Sodium dodecyl-sulfate	85.3 ± 3.6	0.84 ± 0.05
Cetylpyridinium chloride	87.8 ± 2.7	0.85 ± 0.01

[a] Viability of macrophages cultured in medium was considered 100%.

[b] IL-6 levels are shown as mean index IL-6 $\pm$ standard deviation with IL-6 production of untreated cells considered 1.

the particles with a subsequent locally high concentration of toxic degradation products, identical to a locally high concentration of CPC. Our findings are in good agreement with positively charged polymethylmethacrylate particles (by introducing a quarternary ammonium group) showing marked cytotoxicity on various cells (Hoffmann et al., 1997).

We recently presented further evidence that cytotoxicity of SLN may be caused by intracellular degradation of SLN (Schöler et al., submitted). With regard to degradation of SLN, surfactants were shown to influence the degradation profile (Olbrich and Müller, 1999). In comparison, SC as the coating surfactant resulted in accelerated degradation of SLN, whereas, due to their sterical stabilisation, Poloxamer 407 and Tween 80 caused a delayed degradation (Olbrich and Müller, 1999). Furthermore, the amount of biodegradation of SLN has been reported to increase with increasing incubation time. Since we did not observe cytotoxic effects of SLN coated with sterically stabilising surfactants, including P908, P407, P188 and T80, degradation of these SLN appears to be slow and to be complete after 20 h of incubation.

The results presented above are in line with former findings of our group. Stabilising surfactants influenced the extent of cytotoxic effects of SLN on HL 60 cells (Müller et al., 1997b). SLN consisting of Compritol coated with Poloxamer 407 at concentrations of up to 10% did not lead to reduced viabilities of cells, whereas a distinct reduction was observed when using SLN at concentrations of $> 0.1\%$ consisting of the same matrix stabilised with T80 (Müller et al., 1997b). In contrast to our findings in MΦ cultures, viability of HL 60 cells was reduced to 50% when incubated with a solution of Tween 80 alone at concentrations present in 0.0001% SLN. Furthermore, cytotoxicity increased with decreasing molecular weights of Poloxamers (Müller et al., 1997b). Decreased viability at SLN concentrations $> 0.01\%$ was observed using SLN stabilised with Poloxamers 407, 184, 188, 235 or 335. Cytotoxicity was most pronounced for the low molecular weight Poloxamer 188. In addition, SDS-stabilised SLN were well tolerated at concentrations $< 0.01\%$, whereas a solution of SDS (at concentrations present in 0.00001% SLN) exerted distinct cytotoxic effects (Müller et al., 1997b). However, it should be remarked that cytotoxic effects of particulate carrier systems differ, depending on the cell lines used, due to metabolic abilities (e.g. enzymes present) and capabilities of these cell lines (Lherm et al., 1992; Maaßen et al., 1993a,b; Maaßen, 1994; Müller et al., 1997b).

To further study the effect of size on the viability and cytokine production by MΦ, we produced SLN consisting of Dynasan 114 coated with either P188, T80, S75 or CPC ranging in diameters from 0.123 to 2.231 μm (Table 3). Fig. 3 shows that the size of SLN at concentrations of 0.1% did not change their cytotoxic potential on MΦ. As described above, incubation of MΦ with CPC SLN resulted in massive cell death (Fig. 3), whereas P188-, T80- and S75-SLN did not induce major cytotoxic effects. The cytokine production decreased with increasing cytotoxicity (data not shown). These results support results of a previ-

ous study, namely that the size of SLN consisting of stearic acid coated with T80 did not alter cytotoxic effects of SLN (Schöler et al., submitted). Control emulsions — consisting of medium-chained triglycerides and soybean oil (1:1) coated with Lipoid S75 (MCT) — as well as non-degradable polystyrene particles did not exert cytotoxic effects on MΦ at sizes similar to those of SLN (Fig. 3).

Table 3
Diameters of SLN and control emulsion consisting of 10.0% (w/w) lipid coated with 1.0% (w/w) surfactant as measured by laser diffractometry ($n = 3$)

Composition	Abbreviation		Diameter (μm) ± standard deviation	Polydispersity index ± standard deviation
Dynasan 114/Poloxamer 188	P188	Small	0.245 ± 0.127	0.196 ± 0.023
		Medium	0.523 ± 0.211	0.299 ± 0.054
		Large	1.532 ± 0.976	0.456 ± 0.100
Dynasan 114/Tween 80	T80	Small	0.262 ± 0.126	0.107 ± 0.008
		Medium	0.705 ± 1.011	0.202 ± 0.012
		Large	3.191 ± 2.540	0.704 ± 0.124
Dynasan 114/Lipoid S75	S75	Small	0.218 ± 0.1153	0.123 ± 0.0134
		Medium	0.428 ± 0.28	0.305 ± 0.04
		Large	1.430 ± 1.322	0.452 ± 0.117
Dynasan 114/Cetylpyridinium chloride	CPC	Small	0.123 ± 0.058	0.175 ± 0.034
		Medium	0.465 ± 0.123	0.199 ± 0.096
		Large	2.231 ± 1.121	0.487 ± 0.166
Soyaoil/MCT 1:1/Lipoid S75	MCT	Small	0.232 ± 0.124	0.118 ± 0.011
		Medium	0.530 ± 0.429	0.349 ± 0.166
		Large	4.808 ± 1.842	0.523 ± 0.318

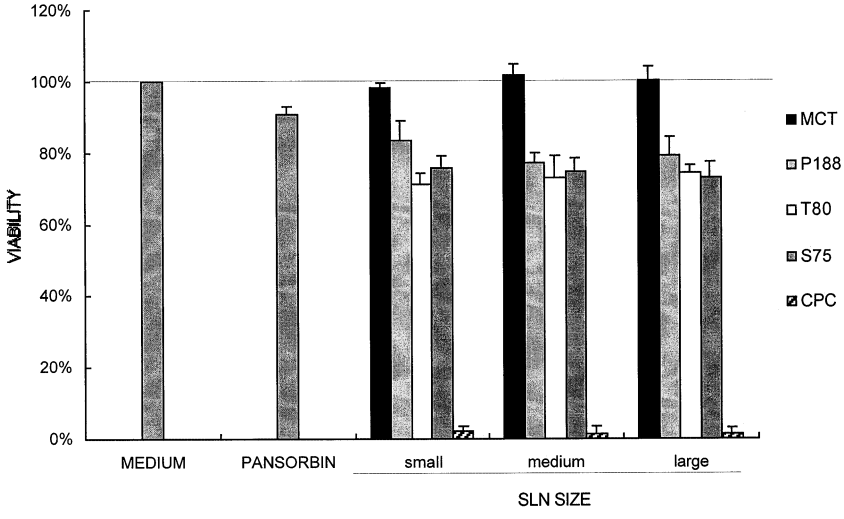

Fig. 3. Viability of peritoneal macrophages after 20 h of in vitro culture with different sized SLN consisting of Dynasan 114 coated with different surfactants at a concentration of 0.1% and with medium, or MCT emulsion (MCT) ($n = 3$, ± standard deviation). Viability of macrophages cultured in medium was considered 100%.

In contrast to our findings, Green et al. (1998) reported that the size of polyethylene particles is a critical factor in the up-regulation of IL-6 and TNF-α production by peritoneal MΦ. The highest concentrations of cytokines were measured in cell supernatants after incubation of MΦ of polyethylene particles in a phagocytosable size (0.3–10 μm). No differences in cell viability were observed after incubation of MΦ with polystyrene particles ranging from 0.2 to 88 μm (Green et al., 1998). In addition, cytotoxic effects of Al_2O_3 particles on the J774 MΦ cell line increased with particle size greater than 2 μm but no IL-6 and TNF-α up-regulating effect could be observed (Catelas et al., 1998).

To investigate macrophage activation, the release of NO was also investigated. No NO was detectable when incubating the cells with aqueous SLN dispersion or pure surfactant solutions. The cells were able to produce NO demonstrated by incubation with Pansorbin leading to an NO level of 11.2 ng/ml.

In conclusion, incubation of MΦ with SLN coated with different surfactants did not result in up-regulation of cytokine production by these cells. Down-regulatory effects were associated with cytotoxicity of SLN. The surfactant, e.g. positively charged molecules, but not the particle size of SLN did influence cytotoxicity and cytokine secretion by MΦ to a great extent. Cytotoxicity and immuno-modulatory effects of SLN should be tested prior to their in vivo use.

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