Solid lipid nanoparticles and microemulsions for drug delivery The CNS.

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CHAPTER 10

Solid lipid nanoparticles and microemulsions for drug delivery: the CNS

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Abstract: The chapter examined solid lipid nanoparticles (SLN) and microemulsions, chosen as carriers of drugs, administered *in vivo* to be transported to the central nervous system. Drugs of different structures and for different therapies have been studied such as doxorubicin SLN stealth and nonstealth administered in rats by intravenous route, apomorphine SLN administered in rats by duodenal route, melatonin SLN administered by transdermal and oral routes in humans, and apomorphine microemulsion administered by transdermal route in Parkinson's patients. The pharmacokinetics of the drug, followed in most studies, put in evidence that the many important pharmacokinetic parameters were notably improved versus the drug alone or in a commercial formulation.

Keywords: solid lipid nanoparticles; microemulsions; drug delivery system; central nervous system

Introduction

The brain homeostasis is of primary importance for survival so that specific interfaces, also referred to as barriers, tightly regulate the exchange between the peripheral blood circulation and the cerebrospinal fluid (CSF) circulatory system. These barriers are represented by the choroid plexus epithelium, the arachnoid epithelium, and the blood–brain barrier (BBB). The concentration and clearance of endogenous and exogenous molecules, essential for the normal brain functions or dangerous because of their toxicity, are strictly regulated by the anatomic and physiologic features of each barrier (Abbott, 2002; Segal, 2000).

The presence of the BBB is certainly the most critical issue encountered in brain drug delivery. Among the possible strategies to deliver therapeutic molecules into the brain, namely, intracerebral, intraventricular, and intravascular delivery, the latest represents the most reliable one because of its potential efficacy, safety, and compliance (Silva, 2007).

Brain capillaries, differently from the peripheral capillaries, present no fenestrae, a low amount of pinocytosis vesicles and particular tight junctions also known zonula occludens. Tight junctions are structures that form a narrow and continuous seal surrounding each endothelial and epithelial cell at the apical border and are at strictly regulating the movements the molecules through the paracellular

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pathway. These structures, together with the brain endothelial cells, make an almost impermeable barrier for drugs administered through the peripheral circulation (Kniesel & Wolburg, 2000; Lapierre, 2000).

A further contribution to the peculiar BBB functions is given by the periendothelial structures represented by astrocytes, pericytes, and the basal membrane (Balahanov & Dore-Duffy, 1998; Lay & Kuo, 2005).

The presence of BBB transport systems further complicates the scenario. In fact, these transporters may assist or hinder the drug delivery to the brain. The carrier-mediated transport may be able to shuttle drugs or prodrugs into the brain in therapeutic concentrations, mimicking nutrients or endogenous compounds (Conford & Hyman, 1999; Pardrige, 1998).

Unfortunately, the presence of active efflux transporters to the BBB also limits the therapeutic efficacy of drugs virtually able to access the brain. The P-glycoprotein (P-gp) is an ATP-dependent drug transport protein present at the apical membranes of different epithelial cell types including those forming the BBB.

Recently, it has been demonstrated, either *in vitro* or *in vivo*, that BBB P-gp can prevent the accumulation of many molecules including a variety of drugs in the brain (Stouch & Gudmundsson, 2002), and P-gp inhibition has been proposed as a possible strategy to enhance the drug penetration (Skinkel, 1999).

Different strategies have been studied for the delivery of drugs to the brain. Indeed most part of the small drug molecules and of large molecules such as recombinant proteins or gene-based molecules are not able to penetrate the BBB and many efforts have been spent in the previous years toward delivery and targeting of drugs to the brain (de Boer & Gaillard, 2007). Many investigations have been carried out in the previous years to improve brain tumors therapy with nanoparticulates; there are less number of studies regarding colloidal carriers of drugs for neurological diseases or of diagnostics. Liposomes, polymeric nanoparticles, and solid lipid nanoparticles (SLN) have been studied, with different approaches, and the problems of overcoming the BBB.

In this chapter, we consider SLN and microemulsions as carriers for the delivery only of drugs active on the central nervous system (CNS). In particular, examining drugs used for therapy in neurological diseases, as many times their administration gives problems, such as high amount of drug administered by parenteral route, short half-life, high hydrophilicity, and poor transport through the BBB. The aim of all the researchers is to study if some improvements in pharmacokinetic parameters in laboratory animals and/or in humans could be achieved using colloidal formulations; the review considers studies on SLN and microemulsions carrying only drugs active on CNS.

Solid lipid nanoparticles

Different approaches are followed for the SLN preparation.

They can be prepared by high-pressure homogenization at elevated or low temperatures, via warm microemulsions, by solvent emulsification–evaporation–diffusion, by high-speed stirring, and/or sonication (Muller, Kader, & Gohla, 2000).

Here we refer only about SLN carrying drugs active on CNS (at brain level).

SLN carrying the lipophilic antipsychotic drug clozapine were prepared by hot homogenization followed by ultrasonication method. Clozapine has a very poor bioavailability (Manjunath & Venkateswarlu, 2005). The SLN were administered by intravenous (IV) and duodenal routes to Swiss albino mice. For the intravenous administration, stearylamine was entrapped with clozapine in SLN; the area under curve (AUC) in the brain increased up to 2.91-fold the one of clozapine suspension.

The same authors (Manjunath & Venkateswarlu, 2006) developed SLN as carriers of the highly lipophilic drug nitrendipine, using different triglycerides for the lipid matrix, soy lecithin, and Poloxamer 188. Positive and negative charged nitrendipine SLN were also produced and then examined to explore the influence of the charge on oral bioavailability. The different kinds of SLN were administered by IV and intraduodenal routes to rats; pharmacokinetic parameters of nitrendipine SLN were examined, tissue distribution studies were carried out in Swiss albino mice, against that of a nitrendipine suspension. Following IV administration nitrendipine-loaded SLN were found to be taken up to a greater extent in tested organs than nitrendipine suspension. The AU3 AUC and MRT of nitrendipine SLN were higher than those of nitrendipine suspension especially in brain and heart. Positively charged SLN were better taken up by the brain and moderately taken up by the heart. Reticuloendothelial system (RES) organs such as liver and spleen were compared with the ones after nitrendipine suspension administration. The higher levels of the drug were maintained AU4 for over 6 h in confront to only 3 h with nitrendipine suspension.

SLN were investigated for their ability to deliver quinine dihydrochloride for the management of cerebral malaria (Gupta, Jain, & Jain, 2007). Quinine was incorporated in SLN and successively coupling of SLN with transferrin (Tf) was achieved by a cross-linker. IV administration of Tf-conjugated SLN enhanced the brain uptake of quinine in confront to the SLN loaded of quinine alone.

In order to enhance the delivery of atazanavir, a HIV protease inhibitor, spherical SLN carrying the drug were tested at first using a well-characterized human brain microvessel endothelial cell line (hCMEC/D3). Cell viability experiments demonstrated that SLN exhibit no toxicity on hCMEC/D3 cells up to a concentration corresponding to 200 nM of the drug. Delivery of ^{3}H-atazanavir by SLN led to a significantly higher accumulation by the endothelial cell monolayer as compared to the drug aqueous solution (Chattopadhyay, Zastre, Wong, Wu, & Bendayan, 2008).

The transport *in situ* of lipid nanoparticles to the brain was evaluated by Koziara, Lockman, Allen, and Mumper (2003); the lipidic nanoparticles were prepared by warm microemulsion precursors followed by hot homogenization technique. Their components were emulsified wax (E wax) or Brij 72 as matrix, and water and Brij 78 as surfactant. The warm microemulsion was cooled upon stirring and the lipid SLN were obtained and homogenized. The SLN were labelled with ^{3}H cetyl alcohol. The transport of the nanoparticles was measured by an "*in situ*" rat brain perfusion method; significant uptake of SLN was obtained . suggesting CNS uptake. The same group studied also the effect that the addition of a thiamine ligand to NPs, obtained by microemulsion as pre- AU5 cursors, causes association with the BBB thiamine transporter (Lockman et al., 2003).

Muller and coworkers studied the preferential adsorption of blood protein onto intravenously injected particulate carriers from different origins (Luck, Paulke, Schroder, Blunk, & Muller, 1998); in particular, Apolipoprotein E (Apo E) on the AU6 surface of P80-coated SLN after their incubation in human plasma citrate. Delivery to the brain using nanoparticulate drug carriers in combination with the targeting principles of "differential protein adsorption" has been proposed (Dehouck et al., 1997). The Pathfinder technology (Muller & Schmidt, 2002) exploits proteins present in the blood which absorb onto the surface of intravenously injected carriers for targeting nanoparticles to the brain. Apo E is one of such targeting molecules for the delivery of nanoparticles to the endothelial cells of the BBB. Apo E can play an important role in the transport of lipoprotein into brain via the low-density lipoprotein receptor present on the BBB. Atoquavone (Muller & Keck, 2004; Scholler et al., 2001) is a drug poorly adsorbed after oral administration, showing poor therapeutic efficacy against toxoplasma encephalitis (TE). Nanocrystals of the drug were produced, their surface was modified with Tween 80 leading to *in vivo* preferential absorption of Apo E; the nanosuspension was IV administered to a murine model of TE, obtaining the disappearance of parasites and of cysts at dose 10-fold smaller than the one of atoquavone administered by oral route.

Solid lipid nanoparticles from warm microemulsions

SLN can be achieved from warm microemulsions.

Warm microemulsions are prepared at temperature ranging from 60°C to 80°C by using AU7 melted lipids (such as triglycerides/fatty acids) as oil, surfactants such as lecithin, and cosurfactants (such as short-chain carboxylates, biliar salts); the warm microemulsions are subsequently dispersed in cold water. The nanodroplets of warm microemulsion, using this procedure, become SLN; they are successively washed by tangential flow filtration. SLN are spherical in shape and with a narrow size distribution .The zeta potential is normally high (30/40 mV) being positive or negative depending on the starting formulation.

Hydrophilic and lipophilic molecules (drugs or diagnostics) can be incorporated in SLN using different methods.

SLN are able to carry drugs of different structure and lipophilicity, such as cyclosporine A (Ugazio, Cavalli, & Gasco, 2002), paclitaxel (Cavalli, Caputo, & Gasco, 2000), doxorubicin (Fundaro, Cavalli, Bargoni, Vighetto, & Gasco, 2000), tobramycin (Cavalli et al., 2003), short-chain fatty acids (Dianzani et al., 2006), peptides (Morel, Cavalli, & Gasco, 1996), antisense oligonucleotides (Brioschi et al., 2008), and melatonin (MT) (Rezzani et al., 2009). Also diagnostic compounds such as iron oxides (Pereira, 2003) have been incorporated into SLN.

SLN can be internalized within 2–3 min into all the tested cell lines (Miglietta, Cavalli, Bocca, Gabriel, & Gasco, 2000; Serpe et al., 2006); administered by duodenal route and are targeted to lymph (Bargoni et al., 1998). SLN stealth can also be prepared to avoid their recognition by the RES, thus prolonging their residence time (Podio, 2001). SLN drug, unloaded or loaded, stealth/or nonstealth, are transported through the BBB (Podio, 2001; Zara et al., 2002).

Drug-loaded solid lipid nanoparticles

In the late 1990s SLN were proposed for brain drug targeting by several groups (Yang, Zhu, Lu, & Liang, 1999; Zara et al., 1999), which studied the pharmacokinetics of two anticancer agents: camptothecin and doxorubicin. After oral and IV administration, they observed drug accumulation into the brain.

Both stealth and nonstealth stearic acid unloaded labelled SLN were found in rat CSF 20 min after IV administration even though low amount of radioactivity was found in the CSF samples collected from cysterna magna (Podio, Zara, Carazzone, Cavalli, & Gasco, 2000).

When the same kind of SLN were loaded with doxorubicin, significantly higher drug concentrations were found in the brain of the animals treated with stealth SLN as compared to nonstealth SLN and doxorubicin solution. The overall plasma pharmacokinetics of stealth and nonstealth SLN provided to be significantly different from that of the doxorubicin solution (Fundaro et al., 2000).

R-apomorphine (10,11-dihydroxyapomorphine) is a well-known potent short-acting dopamine agonist at D1 and D2 dopamine receptors and it was proposed as an antiparkinsonian drug more than a century ago. It significantly reduces the severity and duration of "off" periods and it is able to reverse bradykinesia when administered alone. Despite these favorable clinical effects, the drug's clinical use is somewhat limited by its pharmacokinetic profile: short half-life ($\sim$30 min), rapid clearance from the plasma, lack of storage and retention in brain regions, poor oral bioavailability (5%), and first-pass hepatic metabolism are significant limitations to chronic oral administration. Our group evaluated a new formulation of apomorphine in SLN (submitted data for publishing); the study was designed to investigate the pharmacokinetics and biodistribution of apomorphine incorporated in SLN, injected orally or intravenously in rats.

In vitro the release over time of apomorphine from the SLN dispersion was almost linear After IV administration the peak plasma concentration was higher after apomorphine solution administration than after apomorphine SLN. However, the total area under curve (AUC_{tot}) was nonsignificantly different after SLN than apomorphine solution. The terminal half-life was significantly longer following apomorphine SLN.

Following intraduodenal administration we found that the C_{max} and AUC_{tot} were significantly higher with apomorphine SLN compared to apomorphine solution; on the contrary, the clearance was shorter after apomorphine solution than after the SLN formulation.

In the brain the apomorphine concentration was significantly higher 30 min after apomorphine SLN IV administration versus solution; it was detected only at 4 h after apomorphine SLN injection.

After duodenal administration the drug was detectable in brain only at 30 min after apomorphine SLN administration. No drug was found neither at 4 h nor at 24 h after injection of either apomorphine SLN or the solution.

Furthermore, the free drug concentration was measured in human plasma and we showed that the release started after the absorption of the apomorphine SLN. We also measured the free apomorphine concentration in human blood over time. The amounts in question are relatively low, but may be sufficient to expect clinical effects when administered to parkinsonian patients. After apomorphine solution administration, the amounts of apomorphine determined in the plasma were by far lower than those from SLN, confirming previous studies on the duodenal administration of drug loaded and unloaded SLN (Fig. 1).

In order to furnish a general model for SLN-based delivery systems of drugs devoid of favorable pharmacokinetics, we have recently incorporated MT in SLN (MT-SLN). MT has been chosen for our *in vivo* study because of its safeness in humans even at high dosages.

MT is a hormone produced by the pineal gland at night, involved in the regulation of circadian rhythms. For clinical purposes (mainly disorders of the sleep–wake cycle and insomnia in the elderly), exogenous MT administration should mimic the typical nocturnal endogenous MT levels, but its pharmacokinetics is not favorable due to its short half-life of elimination (DeMuro, Nafziger, Blask, Menhinick, & Bertino, 2000; Mallo et al., 1990). The pharmacokinetics of MT-SLN has been examined in humans after administration by oral and transdermal route (Priano et al., 2007). Three kinds of freeze-dried MT-SLN containing different amounts of MT were prepared and characterized: (a) MT-SLN: MT = 1.8% for *in vitro* experiments (average diameter: 85 nm, polydispersity index = 0.135); (b) MT-SLN: MT = 2% for transdermal application (average diameter = 91 nm, polydispersity index = 0.140); and (c) MT-SLN: MT = 4.13% for oral route (average diameter = 111 nm, polydispersity index = 0.189).

In vitro, MT-SLN produced a flux of MT of 1 μg/h/cm^2 through hairless mice skin, following a pseudo-zero-order kinetics (45). At the same time, AU13 *in vivo* study produced very interesting results,

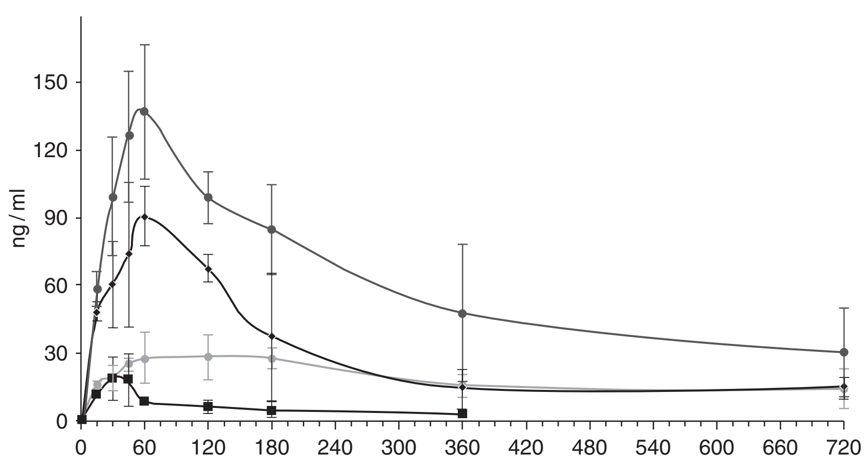

Fig. 1. Plasma levels of free apomorphine and total apomorphine after duodenal administration of apomorphine solution or apomorphine SLN in rats.

confirming in humans that SLN can act as a reservoir that allows a constant and prolonged release of the included drugs (Peira et al., 2003). MT (3 mg) incorporated in SLN was orally administered at 8.30 a.m. to seven healthy subjects; for control purposes, 1 week later the same subjects received orally a standard formulation of MT at the same dose (3 mg) and again at 8.30 a.m. Compared to the MT standard solution, $T_{\max}$ observed after MT-SLN administration was delayed of about 20 min, while mean AUC and mean half-life of elimination were significantly higher (respectively $169{,}944.7 \pm 64{,}954.4$ pg/mL × hour vs. $85{,}148.4 \pm 50{,}642.6$ pg/mL × hour, $p = 0.018$; and 93.1 ± 37.1 min vs. 48.2 ± 8.9 min, $p = 0.009$). Even more, standard formulation and MT-SLN after oral administration produced similar peak plasma levels of MT, even if delayed of about half an hour in the case of MT-SLN. More interestingly, detectable and clinically significant MT plasma levels after MT-SLN oral administration were maintained for a longer period of time, suggesting that SLN orally administered to humans can yield a sustained release of the incorporated drug, a feature that could be particularly useful for molecules, such as MT, characterized by unfavorable kinetics (Priano et al., 2007). Previous studies in laboratory animals indicated a probable targeting of SLN — either drug-loaded or unloaded — to lymph, after duodenal administration (Bargoni et al., 1998). Similarly, the significantly longer half-life of MT observed in the study of Priano et al. (2007) may suggest a targeting of MT-SLN to human lymph, even though the capsules used to administer SLN were not gastro-resistant. In fact, MT half-life of elimination has been calculated in about 40 min after an intravenous bolus and following oral administration low bioavailability and rapid clearance from plasma have been shown, primarily due to a marked first-pass hepatic metabolism. Moreover, pharmacokinetic analysis following transdermal administration of MT-SLN demonstrated that plasma levels of MT similar to those produced by oral administration may be achieved for more than 24 h (50). In 10 healthy subjects, SLN incorporating MT were administered transdermally by applying a patch at 8.30 a.m. and leaving it in place for 24 h. In this delivery system, MT absorption and elimination were slow (mean half-life of absorption = 5.3 ± 1.3 h; mean half-life of elimination = 24.6 ± 12.0 h) so that MT plasma levels above 50 pg/mL were maintained for at least 24 h (Figs. 2 and 3). Tolerability of MT-SLN administered transdermally or by oral

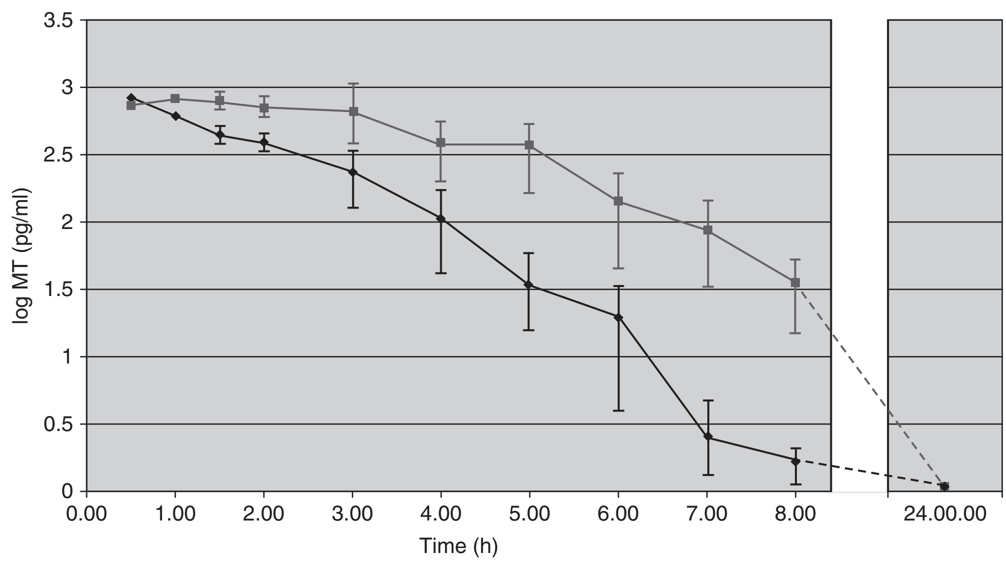

Fig. 2. MT plasma profile in humans after MT (♦) and MT-SLN (■) oral administration.

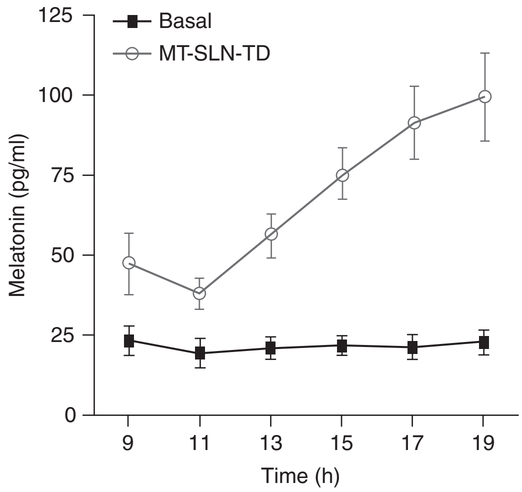

Fig. 3. MT plasma levels in humans at baseline and after MT-SLN transdermal administration (MT-SLN-TD).

route was good and no adverse effect occurred, apart from a predictable mild somnolence and transient erythema after gel application. This means that, at least at the doses used in that study (45), SLN administration via the oral or transdermal routes is safe.

In this context, we also tested transdermal MT-SLN for three consecutive nights in five patients suffering from delayed sleep phase syndrome (unpublished data), confirming the safeness of this formulation. Due to the small sample, however, the tendency of clinical benefits was present but statistical significance could not be reached, so that further investigations in larger samples are needed in order to evaluate the impact of this new formulation in clinical practice.

However, these very favorable results, obtained in humans administering MT-loaded SLN, clearly suggest that SLN can be considered effective *in vivo* delivery systems that could be suitably applied to different drugs, and in particular to those requiring prolonged high plasma levels but that have unfavorable pharmacokinetics. Finally, it must be stressed that, since doses and concentrations of drugs included in SLN can be varied, different plasma level profiles could be obtained, thus disclosing new chances for sustained delivery systems adaptable to a variety of clinical conditions (Priano et al., 2007).

Suitability of SLN to convey drugs into CNS is also confirmed by studies regarding baclofen included in SLN. Intrathecal baclofen administration represents the reference treatment for spasticity of spinal or cerebral origin. Nevertheless, surgical involvement together with risk of infection or catheter dysfunction may limit the number of potentially treatable patients (Dario & Tomei, 2004; Perot & Almeida-Silveira, 1994). In order to explore alternative and efficacious routes of administration, we studied a new pharmaceutical preparation characterized by SLN incorporating baclofen (baclofen-SLN) (submitted data for publishing). Baclofen concentration, after reconstitution with water of freeze-dried SLN, was 1.7 mg/mL. Groups of Wistar rats were injected intraperitoneally with physiological solution and unloaded SLN at 10 mL/kg (control groups), with baclofen-SLN (baclofen-SLN group), and baclofen solution (baclofen-sol group) at increasing dosages of 2.5, 5, 7.5, 8.5, and 10 mg/kg. At different times up to the fourth hour, efficacy testing was performed by means of H-reflex, while behavioral characterization was obtained using two scales validated for motor symptoms due to spinal lesions and sedation in rat models (Nemethy, Paroli, Williams-Russo, & Blanck, 2002; Tsunoda, Kuang, Tolley, Whitton, & Fujinami, 1998). Rats were sacrificed for detecting baclofen concentration in blood and tissue. Compared to baclofen-sol and control group, *H*/*M* amplitude curve after baclofen-SLN injection was characterized by a dose-dependent reduction at the first and second hours, so confirming efficacy, and a rebound increase at the fourth hour, indicating an unexpected belated spinal hyperexcitability (Fig. 4). Similarly, baclofen-SLN effect on behavioral scales was stronger compared to baclofen-sol group, with the maximum effects obtained at the first hour. Moreover, clinical effects were detectable after low dosages of baclofen-SLN (2.5 mg/kg) but only after higher dosages of baclofen-sol (7.5 mg/kg). After 4 h from the injection, only the rats treated with the higher dosages of baclofen-SLN still presented clinical signs consisting in sedation (8.5 mg/kg) or complete paralysis and piloerection (10 mg/kg). On the whole, these data suggest a dose-dependent modulation of spinal reflex excitability, which is

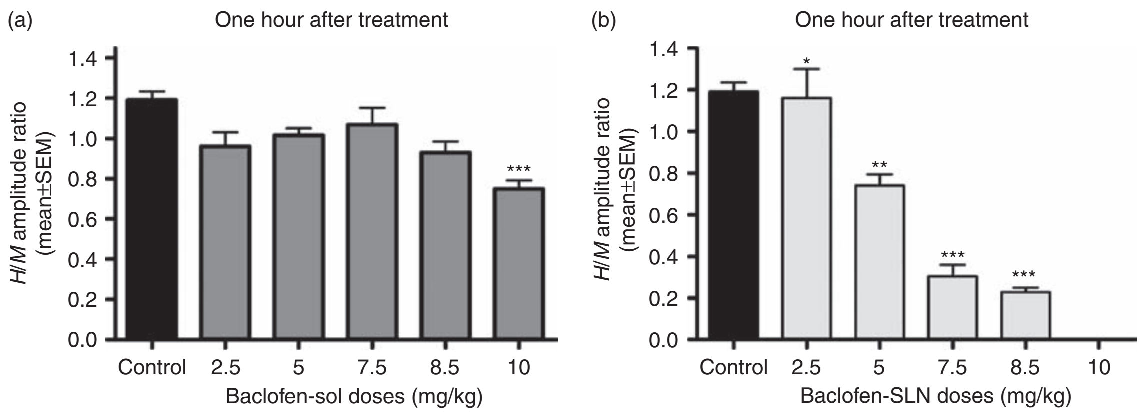

Fig. 4. H/M amplitude ratios after baclofen-solution (a) and after baclofen-SLN (b), at increasing doses, compared to control animal group. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

not so evident after administration of standard formulation of baclofen. Nevertheless, important cortical effects were also present. Clinical data were related with plasma and tissue concentrations. In fact, after 2 and 4 h only baclofen-SLN administration produced measurable baclofen plasma concentrations, with an almost linear decrease of baclofen appreciable for 4 h. On the contrary, undetectable amount of baclofen in plasma were noticed 2 h after administration of baclofen-sol. In brain, both the two formulations (baclofen in solution and in SLN) gave a maximum after 2 h but concentrations after SLN were almost twice the ones after solution. This last data might be due partly to the free drug already released and to baclofen-SLN overcoming the BBB. We realize that for clinical purposes this AU15 effect of baclofen-SLN is unwished, as it is responsible for sedation. However, baclofen-sol injections also produced sedation, even if weaker and corresponding to lower plasma concentrations, compared to baclofen-SLN. In conclusion, higher spinal and cortical effects of baclofen-SLN, compared to equivalent dosages of baclofen-sol, seem attributable to higher and more prolonged concentrations of drugs in plasma and brain.

As previously noted, unloaded SLN administered by duodenal route are targeted to lymph and the incorporated drug can be partly distributed in the brain; moreover, SLN can also be prepared stealth for increasing their residence time (Bargoni et al., 1998; Fundaro et al., 2000; Podio, 2001; Zara et al., 2002). Other new studies will be directed toward a duodenal administration of baclofen-SLN stealth, not only for prolonging their residence time but also to target them to lymph, enhancing their bioavailability. Further research should also be directed toward the optimization of dosages and concentrations of baclofen included in SLN, in order to preserve the prolonged antispastic effect, peculiar of this new formulation, but devoid of clinically significant cortical effects.

Solid lipid nanoparticles as potential diagnostics

Superparamagnetic iron oxides are classified as contrast agents for magnetic resonance imaging (MRI). They are able to affect the water relaxation times T_1 and T_2; their ability in altering such properties is quantified by the parameter relaxivity. Iron oxides are able to affect preferentially the T_2 relaxation times of tissues (and are called T_2-relaxing agents) while paramagnetic contrast agents such as Gd complexes affect mainly T_1 and are called T_1-relaxing agents.

Iron oxides are insoluble in water; therefore, to be clinically used they must be transformed in modified colloids while their magnetic properties should remain unchanged. The surface of the iron oxide nanoparticles can be modified, covering them by hydrophilic macromolecules; such as dextran in the case of Endorem.

A research was performed in order to know whether SLN can load iron oxides and whether they are able to reach the brain. Two different AU16 SLN, SLN-Fe^{A} and SLN^{B} containing iron oxides were prepared from warm microemulsions and studied at first *in vitro* (1). The comparison of Fe-SLN was performed with Endorem. Both the Fe-SLN preparations showed relaxometric properties similar to the ones of Endorem. The good T_2-relaxation-enhancing properties allow an *in vivo* study of their distribution by MRI. Fe-SLN^{B}, at the higher Fe concentration, were administered IV to rats; the comparison was performed with Endorem. Images obtained after Endorem IV administration show early modification, but soon return to baseline; these findings are consistent with short Endorem retention time in blood. Results from SLN-Fe^{B} show a different behavior. For each part of the AU17 brain, maximal SS is reached in the last images (135 min after administration). SS increase from the first to the last acquisition. This study shows that after inclusion in SLN, Endorem becomes a new type of contrast agent: Endorem is taken by the liver and does not cross the BBB, while Endorem containing SLN-Fe^{B} shows CNS uptake. This means that SLN-Fe kinesis is related to SLN and not to their iron oxide content as already seen.

Microemulsions

Microemulsions are transparent, thermodynamically stable dispersions of water and oil, usually stabilized by a surfactant and a cosurfactant. They contain particles smaller than 0.1 μm. Microemulsions are often defined as thermodynamically stable liquid solutions; the stability of microemulsions is a consequence of the ultralow interfacial tension between the oil and water phases. A clear distinction exists between microemulsion and coarse emulsions. The latter are thermodynamically unstable, droplets of their dispersed phase are generally larger than 0.1 μm and consequently their appearance is normally milky rather than transparent.

The limits in the use of microemulsions in the pharmaceutical field are chiefly from the need of all the components to be acceptable, particularly surfactants and cosurfactants — the amounts of surfactants and cosurfactants required to form microemulsions are usually higher than those required for emulsions.

Recently, apomorphine was incorporated into microemulsions to study whether they are a feasible vehicle for transdermal transport of this drug. In the preparatory *in vitro* study (Peira, Scolari, & Gasco, 2001), two different microemulsions whose components were all biocompatible were studied: the concentration of apomorphine was 3.9% in each. Since apomorphine is highly hydrophilic, to increase its lipophilicity, apomorphine–octanoic acid ion pairs were formed. At pH 6.0, log P_{app} of apomorphine increased from 0.3 in the absence of octanoic acid to log $P_{app} = 2.77$ for a molar ratio 1:2.5 (apomorphine: octanoic acid). The flux of drug from the two thickened microemulsions through hairless mouse skin was, respectively, 100 and 88 μg/h/cm^2. The first formulation, having the higher flux, was chosen for *in vivo* administration to Parkinson's patients.

For the *in vivo* study, 21 patients with idiopathic Parkinson's disease who presented long-term L-DOPA syndrome, motor fluctuations and prolonged "off" periods were selected (Priano et al., 2004). Here, 10 g of apomorphine hydrochloride (3.9%), included in microemulsion for transdermal delivery (Apo-MTD), was applied to a 100 cm^2 skin area on the chest; the area was delimited by 1-mm-thick biocompatible foam tape and covered with a polyester-based membrane and an occlusive membrane to prevent evaporation. In these conditions, a single layer of microemulsion (1 mm thick) was directly in contact with the skin surface and acted as a reservoir of apomorphine. Apo-MTD was applied at 8.00 a.m. and left for 12 h. In all patients, except two, apomorphine was detected in blood samples after a variable lag time. Pharmacokinetic analysis revealed that epicutaneous–transdermal apomorphine absorption was rapid (mean half-life of absorption = 1.03 h) with a variability among patients (half-life of absorption, SD = 1.39 h). Mean $C_{\max}$ was above the therapeutic range (mean $C_{\max}$ = 42.81 ± 11.67 ng/mL), with a mean $T_{\max}$ of 5.1 ± 2.24 h. Therapeutic concentrations of apomorphine were reached after a mean latency of 45 min (range 18–125), and stable concentrations, above the therapeutic range, continued for as long as Apo-MTD was maintained in place. At the 12th hour, Apo-MTD was removed, and the apomorphine plasma concentration then decreased at a rate comparable to that described for subcutaneous administration (mean half-life of elimination equal to 10.8 ± 1.93 h). $C_{\max}$ and AUC showed good correlations with the reduction of "off" periods duration and with the improvement of clinical scores evaluating motor performances (*r* values ranging form 0.49 to 0.56, with *p* values ranging from 0.02 to 0.04). Apo-MTD overall tolerability was good: systemic side effects were similar to those caused by subcutaneous apomorphine injection (sleepiness, mild orthostatic hypotension, and transient nausea), and in the case of nausea, they were strictly related to the highest plasma level of apomorphine. Moreover, regarding local side effects, the large majority of patients (71.4%) presented a transient mild erythema at the site of Apo-MTD application, with a complete regression within 48 h, whereas only in two cases the erythema lasted more than 3 days and required local therapy. This study clearly demonstrated that in most Parkinson's patients Apo-MTD is absorbed by the epicutaneous–transdermal route. This result is in contrast with other reports, where the transdermal route did not produce detectable plasma levels of apomorphine, or in which no apomorphine was transported passively through the skin (Gancher, Nutt, & Woodward, 1991; van der Geest, van Laar, Gubbens-Stibbe, Boddé, & Danhof, 1997). Probably, this difference was mainly due to the peculiar pharmaceutical preparation used. Even if pharmacokinetic parameters are variable, Apo-MTD demonstrated the feasibility of providing therapeutic apomorphine plasma levels for much longer periods of time than previously tested apomorphine preparations (several hours), allowing a more constant dopaminergic stimulation. These results are encouraging and Apo-MTD might become of clinical value in some parkinsonian patients suffering from uncontrolled "wearing-off" and prolonged "off" phenomena. On the contrary, because of the lag time of about 1 h before therapeutic concentrations are reached, Apo-MTD may not be the "ideal" preparation for rapid relief of "off" periods.

Since Apo-MTD was found to provide constant drug release over several hours, other studies have been addressed to its use for the nocturnal sleep disorders of Parkinson's patients. Twelve parkinsonian patients underwent standard polysomnography on basal condition and during one night treatment with Apo-MTD (applied to 100 cm^2 from 10 p.m. until 8 a.m.; Priano et al., 2003). Sleep analysis during APO-MTD treatment in comparison to basal condition showed very favorable findings: 16% increment of total sleep time, 12% increment of sleep efficiency, 16% increment of stage 3 and 4 nonrapid eye movement (NREM), 15% reduction of periodic limb movements index, 22% reduction of arousal index, and 23% reduction of the "cycling alternating pattern" rate, an objective measure of disruption and fragmentation of NREM sleep. Pharmacokinetic analysis confirmed the absorption of apomorphine and the maintenance of therapeutic plasma levels for several hours (mean $C_{\max}$ = 31.8 ± 9.7 ng/mL; mean $T_{\max}$ = 3.1 ± 1.6 h; mean half-life of absorption = 1.2 ± 1.4 h; mean half-life of elimination = 8.8 ± 1.9 h). On the whole, this study confirmed that APO-MTD in Parkinson's disease might be able to reduce nocturnal anomalous movements, akinesia, and rigidity, and might be efficacious for reducing the instability of sleep maintenance typical of parkinsonian sleep.

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