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Preparation and characterization of liposomal formulations of neurotensin-degrading enzyme inhibitors

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ABSTRACT

Neurotensin-degrading enzyme (NTDE) inhibitors hold great potential for treating psychotic disorders. However, brain uptake of such compounds *in vivo* is generally low due to the presence of the blood–brain barrier. In this study, liposomal formulations of two NTDE inhibitors, named compound 1 (C1) and compound 2 (C2) were prepared. Association of these compounds with the liposomal bilayer, subsequent liposomal stability, and compound release in the presence of albumin was studied. Entrapment of the compounds in the liposomal bilayer showed the solubilizing properties of the liposomes. Size and polydispersity index of the compound-entrapped liposomes did not change over 1 month, showing colloidal stability of the liposomal drug formulations. The amount of compounds associated with the liposomes decreased within one day. After this, the association remained stable at 4 °C. For C1, association remained stable at 37 °C in HEPES buffered saline, and the compound was gradually released in the presence of bovine serum albumin. For C2, the release was rapid in both HBS and BSA at 37 °C. In conclusion, the formulation of NTDE inhibitors C1 and C2 in liposomes has been demonstrated and holds promise to deliver NTDE inhibitors *in vivo*.

1. Introduction

Neurotensin is a tridecapeptide found within the central nervous system (CNS) and the gastrointestinal tract. Three main neurotensin receptors have been identified (NTS1, NTS2, and NTS3). In the gastrointestinal tract, neurotensin is involved in gut motility. In the brain, it is highly expressed in the limbic system, and is involved as a neurotransmitter in many processes, including antinociception, hypothermia, control of anterior pituitary hormone secretion, and muscle relaxation (Boules et al., 2006). In addition, neurotensin acts as a neuromodulator affecting the dopaminergic pathways in the brain. Dopamine receptors are divided into the D1 and D2 subfamilies, and neurotensin antagonizes dopamine effects at the D2 receptors. Neuronal D2 receptors are located on both the presynaptic and the postsynaptic site. At the presynaptic site, D2 regulates synthesis and release of dopamine. Postsynaptically, D2 inhibits downstream effects of dopamine, by inhibiting adenylyl cyclase and the cAMP pathway (De Mei et al., 2009). Presynaptic neurotensin antagonism of D2 receptors leads to an increase of dopamine release, enhancing postsynaptic dopamine D2 action. In contrast, postsynaptic neurotensin antagonism of D2 receptors results in a reduction of dopamine D2 neurotransmission (Caceda et al., 2006). This resembles the action of antipsychotic drugs, which block D2 receptors (Seeman, 2010), making neurotensin a possible endogenous antipsychotic (Caceda et al., 2006; Kinkead and Nemeroff, 2002). Because of the close interaction of neurotensin and the dopamine pathways, neurotensin may be involved in many dopamine-related disorders, such as Huntington's disease, Parkinson's disease, and drug abuse. For this reason, modulating the concentration of neurotensin in the brain is of therapeutic interest, and several neurotensin analogs have been developed (Boules et al., 2006).

When neurotransmitters and neuropeptides are released from the cells that store them, they are meant to produce rapid peak signals that last only shortly. Therefore, after release, neurotensin is cleaved and inactivated by metallo-endopeptidases in the extracellular space near the target cells. There are 3 major neurotensin-degrading enzymes: EP 24.11, EP 24.15, and EP 24.16. Their cleavage sites are indicated in Fig. 1. Cleavage by these enzymes leads to complete biological inactivation of neurotensin (Kitabgi, 2006). In order to prevent neurotensin degradation, NTDE inhibitors have been synthesized. This approach is an alternative to the use of neurotensin analogs, and is meant to increase the effects of endogenous neurotensin. *In vivo* experiments have shown that an intracerebroventricularly (icv) injected mixed inhibitor of all three major NTDEs was able to potentiate the hypothermic effects

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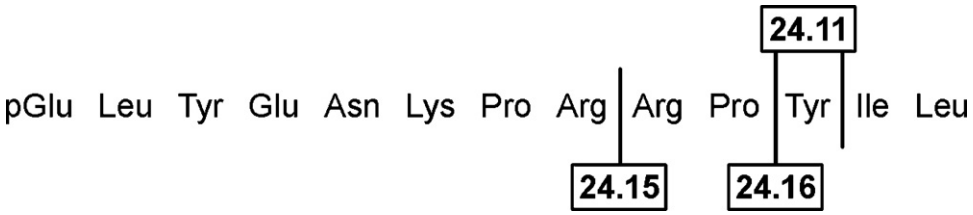


Fig. 1. Neurotensin amino acid sequence and cleavage sites for endopeptidases EP 24.11, EP 24.15, and EP 24.16.

of icv injected neurotensin (Doulut et al., 1993). In another study, an icv injected inhibitor selective for EP 24.25 and EP 24.16 was able to potentiate the antinociceptive effects of icv injected neurotensin (Vincent et al., 1995).

The *in vivo* experiments performed with NTDE inhibitors only showed effects when the drug was administered by icv injection. Lack of sufficient effect after intravenous administration is due to the blood–brain barrier (BBB). This barrier is formed by the brain endothelial cells that surround the microvessels of the brain. Additionally, other cell types surrounding the endothelium, such as astrocytes and pericytes, contribute to the formation of the BBB (de Boer et al., 2003). Due to the tightness and strict transport regulation of the barrier, less than 98% of small molecule drugs can enter the brain (Pardridge, 2007). For clinical applications, icv injection is not an option (de Boer and Gaillard, 2007). Therefore, a drug delivery vehicle is needed to transport peripherally administered NTDE inhibitors to the brain. Liposomes have been widely used as delivery vehicles to increase uptake into the brain *in vivo* (Afergan et al., 2008; Schnyder et al., 2005; Shi et al., 2001; Xie et al., 2005). By coupling specific BBB targeting ligands to the outside of the liposomes, they can enter the brain endothelial cells through receptor-mediated endocytosis, followed by transcytosis to the brain (van Rooy et al., 2010a).

In this study, liposomal formulations of two 2,3-diarylpyparazolidine derivatives, named compound 1 (C1) and compound 2 (C2) (Feenstra et al., 2003) were prepared. These lipophilic compounds act as neurotensin-degrading enzyme (NTDE) inhibitors in the brain, but show poor brain uptake *in vivo*. Association of C1 and C2 with liposomes, subsequent liposomal stability, and compound release in the presence of albumin was studied. The structural formulas and characteristics of C1 and C2 are shown in Table 1.

2. Materials and methods

2.1. Materials

Cholesterol was obtained from Sigma (St. Louis, MO). Dipalmitoyl phosphatidylcholine (DPPC) and distearoyl phosphatidylethanolamine–polyethyleneglycol$_{2000}$ (DSPE–PEG$_{2000}$) were obtained from Lipoid GmbH (Ludwigshafen, Germany). Compounds 1 and 2 were obtained from Abbott Healthcare Products B.V. (formerly Solvay Pharmaceuticals B.V., Weesp, The Netherlands).

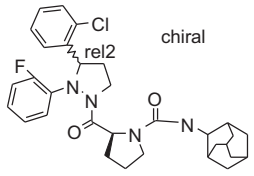
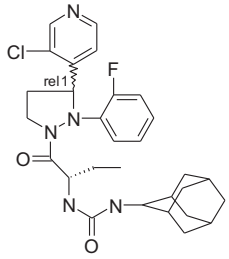
2.2. HPLC detection of compounds

Drugs were dissolved in pure ethanol to yield 10 mg/ml stock solutions. UV–vis absorption at a wavelength range from 220 to 400 nm determined the optimal detection wavelength at 233 nm for both compounds. Compound concentration determinations were performed by high-performance liquid chromatography (HPLC, Waters corporation, Milford, MA), using a C18, 4.6 mm × 150 mm column (Sunfire), at a flow rate of 1 ml/min. Gradient mobile phase consisted of solvent A (methanol:H_2O 5:95) and solvent B (methanol), and was changed during 15 min from 50/50 solvent A/B to 100% solvent B, and ran additionally for 3 min at 100% solvent B. Both solvents contained 0.1% perchloric acid. UV detection was performed at 233 nm. Compounds showed a retention time of 12 min (C2) and 15 min (C1). HPLC sample injection volume was 50 μl and for liposomes proper sample dilutions were made to obtain a concentration estimated to fit within a free drug standard curve.

2.3. Liposome preparation

Liposomes were prepared by the film hydration method. Lipids (DPPC, cholesterol and DSPE-PEG$_{2000}$) were dissolved in chloro-

Table 1
Structural formulas and characteristics of NTDE inhibitors C1 and C2 (Feenstra et al., 2003). Characteristics are based on unpublished results.

Name	Structural formula	MW(Da)	IC_{50} (nM)[a]	AlogP	Oral bioavailability (%)	CNS/plasma ratio
C1		551	100	5.9	0.7	0.2–0.5
C2		540	20	4.9	18	0.02

[a] IC_{50} values were obtained according to the methods described in (Dauch et al., 1991).

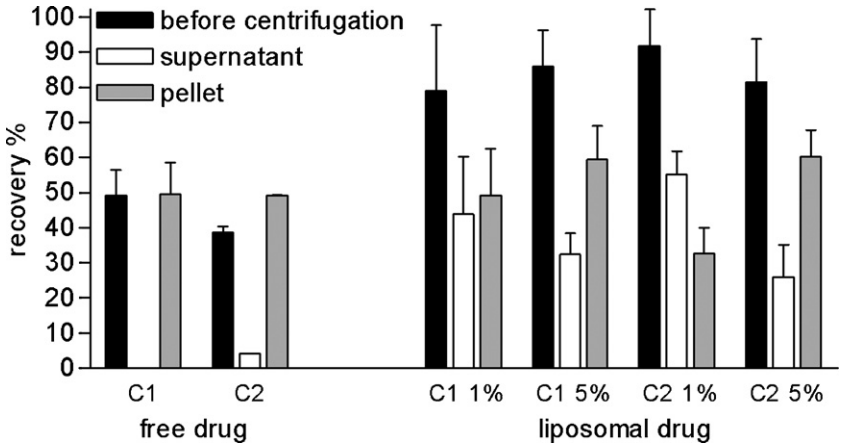


Fig. 2. Recovery percentage of compounds C1 and C2 from free and liposomal drug suspensions. In the liposomal formulations, the amount of drugs added was 1 or 5 mol% of total lipid. The total amount of drugs added to the suspensions was set at 100%. Concentrations were determined by HPLC before and after centrifugation. After centrifugation (10,000 × *g*, 10 min), the amount of drug was determined in the pellet (precipitated drug) and supernatant (free or liposomal drug in suspension). Mean ± s.d. $n = 3$.

form, in a 1.85:1:0.15 molar ratio. Compounds were mixed with the lipid solution to achieve a final compound concentration of either 1 mol% or 5 mol% of total lipids. A lipid film was made by rotary evaporation. The lipid film was hydrated in HBS (10 mM HEPES, 146 mM NaCl, pH 7.4) to a final lipid concentration of 30 μmol/ml. Liposomes were sized by probe sonication at 100% amplitude, 0.5 cycles for 20 min on ice (Labsonic P, 50/60 Hz, Satorius). To remove drugs that did not associate with the liposomes, the liposome solution was centrifuged at 20,000 × *g* for 10 min. Supernatant containing the liposomes was collected. Precipitated drug pellet was resuspended in ethanol. A phosphate determination (Rouser et al., 1970) of both the pellet and the supernatant confirmed that all liposomes were in the supernatant, and no liposomes were present in the pellet. Both pellet and supernatant were subjected to HPLC for compound determination.

2.4. Characterization of liposomes

The mean particle size distribution (*z*-average) and the polydispersity index of the liposomes were determined by dynamic light scattering (DLS) in a Malvern ALV CGS-3 (Malvern Instruments, Malvern, UK) containing a He–Ne laser source (λ = 632.8 nm, 22 mW output power) under an angle of 90°. Measurements were performed in HBS (10 mM HEPES, 146 mM NaCl, pH 7.4). When size distribution and polydispersity index were measured over time, liposomes were subjected to centrifugation at 20,000 × *g* for 10 min before each measurement.

2.5. Compound association at 4 °C

Liposomes were stored at 4 °C. At various time points, liposomes were centrifuged at 20,000 × *g* for 10 min. Drug concentrations in both liposomal supernatant and free drug pellet were measured by HPLC. The total amount of drugs added to the liposomes was set at 100%.

2.6. Compound leakage at 37 °C

Drug release from liposomes was studied over 7 days at 37 °C, in the presence of either HBS or BSA, as modified from Peschka et al. (1998). One day old liposomes (30 μmol/ml lipid) were centrifuged at 20,000 × *g* for 10 min to remove unassociated drugs. 2% of agarose was dissolved in water and liquefied by heating. Equal volumes (250 μl) of agarose solution and liposomes were mixed in glass vials. After this layer solidified, a second layer of 2% agarose (150 μl) was added on top. A receptor solution of 4.5 ml HBS (10 mM HEPES, 146 mM NaCl, pH 7.4), or 4.5 ml BSA in HBS (40 mg/ml) was poured on top. In this way, the agarose-embedded liposomes were physically separated from the buffer by the 2% agarose layer. The liposomes were trapped in the agarose, while the HBS and albumin were able to penetrate into it. The vials were incubated at 37 °C while shaking at 250 rpm, and the receptor solution was replaced at various time points. The concentration of compound in the replaced solutions was determined by HPLC. The amount of drugs in the liposomes after centrifugation (before agarose embedding) was set at 100%. Peschka et al. showed that 98.7% of liposomes remained intact after 5 days using this method (Peschka et al., 1998).

3. Results

3.1. Preparation of liposomal drug formulations

The solubility of NTDE inhibitors C1 and C2 in aqueous buffer is extremely poor. To determine whether encapsulation in a liposomal bilayer could increase the solubility, suspensions of both free and liposomal drugs in aqueous buffer (HBS) were prepared. Liposomes were composed of phosphatidylcholine, cholesterol and DSPE-PEG_{2000}, as this liposomal composition has been used before to target to the brain (Gosk et al., 2004; Schnyder et al., 2005; Xie et al., 2005; Zhang et al., 2004). After preparation, liposomal suspensions were centrifuged to spin down non-dissolved drugs, and the amount of drugs in pellet and supernatant was determined. As shown in Fig. 2, about 50% of the free C1 and C2 in the suspension could be recovered. This was probably due to the highly lipophilic nature of both compounds, which makes them stick to polypropylene disposables used for preparation and detection (Pan et al., 2001). When the free drug suspension was centrifuged, all of C1 and most of C2 was pelleted, underlining the poor solubility. When liposomal formulations of these drugs were prepared, a much higher amount of drug could be recovered from the suspension, showing the solubilizing properties of the liposomes. When the liposomes were centrifuged, a high percentage of free drug was pelleted, indicating that not all of the solubilized drug had been encapsulated inside the liposomes. The total association percentage was 25–55%, depending on the compound concentration, indicating that there is an association plateau.

3.2. Characterization and stability of liposomal drug formulations

Stability of the liposomal formulations in terms of size, polydispersity, and drug association was monitored over a one month

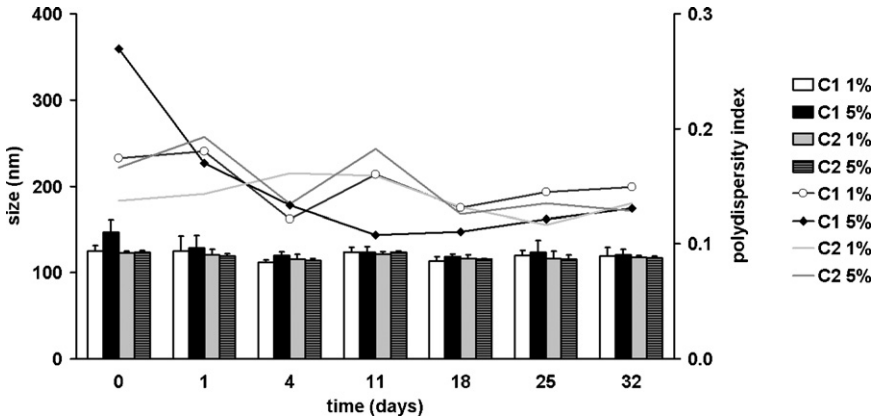


Fig. 3. Size (bars) and polydispersity index (lines) of liposomal preparations of compounds C1 and C2, measured over a period of 32 days while liposomes were stored at 4 °C. The amount of drug added to the liposomes was 1 or 5 mol% of total lipid.

period. During this time the liposomes were stored at 4 °C. As shown in Fig. 3, the average size of all four liposomal formulations was stable at around 130–140 nm. The polydispersity index (PDI) was in the range of 0.1–0.2.

The release of compounds C1 and C2 from these liposomes was determined over one month. As shown in Fig. 4, both C1 and C2 showed an initial burst release of the compounds within the first 24 h. After this release, the association of the drugs with the liposomes stabilized.

3.3. Drug release at 37 °C in the presence of albumin

Drug release of compounds C1 and C2 from the liposomes was studied at 37 °C in the presence of buffer (HBS), with or without albumin. Albumin is the most abundant plasma protein and is known to interact with many drugs, due to its hydrophobic pockets (Kratz, 2008). To study the release of the compounds in the presence of HBS and BSA, one day old liposomes were centrifuged to remove unassociated drugs, and were embedded in a 1% agarose gel, and covered by a physical protection layer of 2% agarose gel. A receptor solution consisting of either HBS or BSA solution was poured on top. The solution was replaced at several time points during 7 days. The drug concentration in the replaced solution was measured. Fig. 5 shows the cumulative release of the drugs from the liposomes into the receptor solutions. C1 was not released in HBS during the time period measured (Fig. 5A), which was comparable to the previous experiment at 4 °C (Fig. 4A). When liposomes were incubated with BSA solution, release of C1 was first measured after 8 h, and continued gradually over 7 days. For compound C2, release

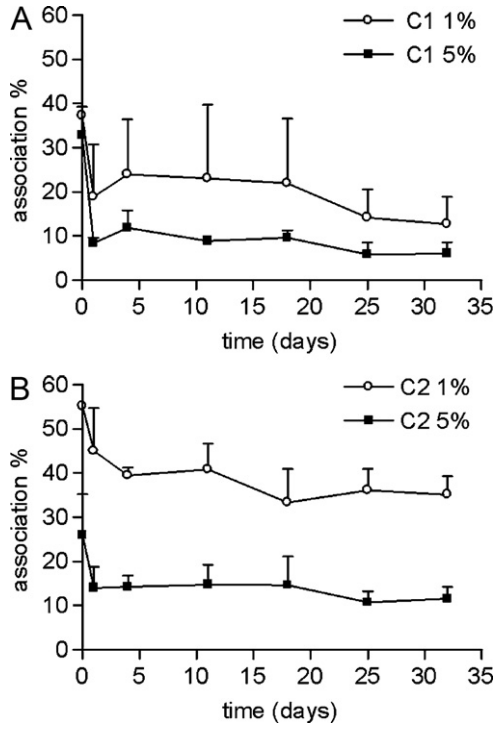


Fig. 4. Association percentage of compounds C1 (A) and C2 (B) to liposomes, measured over a period of 32 days while liposomes were stored at 4 °C in HBS buffer. The total amount of drugs added to the liposomes was set at 100%. The added drug amount was 1 or 5 mol% of total lipid. The percentage of drugs associated with the liposomes was measured by HPLC. Mean ± s.d. $n = 3$.

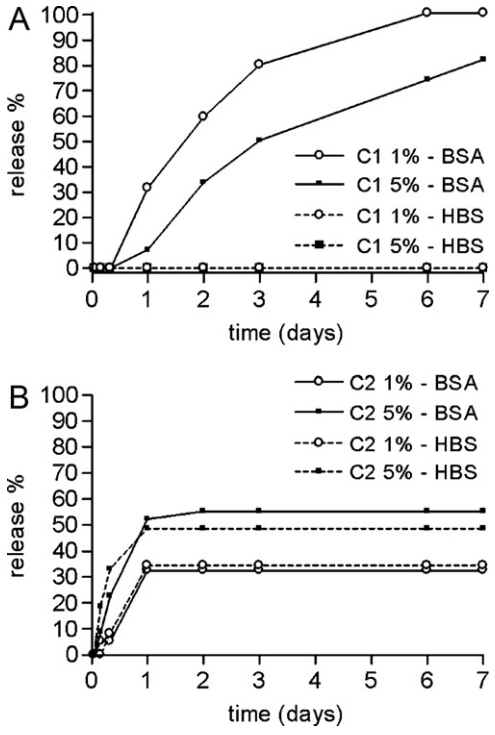


Fig. 5. Cumulative dissociation of compounds C1 (A) and C2 (B) from liposomes embedded in agarose, measured over a period of 7 days while liposomes were shaken at 37 °C. The amount of drugs in 1 day old liposomes (after centrifugation, before agarose embedding) was set at 100%. The added drug amount was 1 or 5 mol% of total lipid. Dissociation was measured in either HBS or BSA. $t = 0$ is the time point of embedding.

was detected at the first time point measured (1 h), regardless of the receptor solution composition (Fig. 5B), implicating that the release of C2 was not related to protein interaction. Release of C2 reached a maximum within 1 day. Although only 30–50% of C2 could be recovered, this probably represents 100% of the drug, as the remaining 50–70% was not found within the agarose gel after 7 days. Again, the lipophilic nature of the compound probably resulted in loss of the compound during preparation and measurement.

4. Discussion

Liposomal formulations of two NTDE inhibiting compounds with poor aqueous solubility were prepared. Although entrapping these compounds in the bilayer of liposomes clearly increased the solubility of these compounds, the association efficiency of C1 and C2 decreased after 1 day, resulting in a low final amount of drugs associated to the liposomes. The reason for the rapid dissociation of the compounds remains unclear. It is known that lipophilic compounds are able to destabilize the bilayer and can lead to the disintegration of the liposomes (McCormack and Gregoriadis, 1994). However, size and polydispersity index of the liposomes remained stable. Possibly the drug aggregated and precipitated.

After the initial release, the liposomal association of C1 in HBS remained stable. In conditions mimicking the circulation (a 37 °C shaking BSA solution), C1 release commenced after 8 h, and the compound was gradually released over 7 days. This is of interest for the *in vivo* situation since a substantial part of these type of liposomes will be taken up into the brain within 8 h after iv administration, and the majority will usually be taken up within 24 h (Paolino et al., 2010; Wu et al., 2006). Drug release from the carrier at the target site (brain) is of major importance (Lindner and Hossann, 2010). Therefore, a liposome gradually releasing the drug could be an interesting vehicle for delivering the NTDE inhibitor to the brain. For compound C2, release under these conditions started immediately. Therefore, this formulation is probably less suitable for *in vivo* applications.

To induce uptake of C1 liposomes into the brain, they should preferentially be coupled to a brain-targeting ligand. For example, anti-transferrin receptor antibodies such as OX26 or RI7217 are good candidates for liposomal targeting to the brain (Gosk et al., 2004; van Rooy et al., 2010b). Once the ligands bind to the brain endothelial cells, the liposomes can be taken up by receptor-mediated endocytosis, and release their contents (Pardridge, 2005).

In conclusion, we have demonstrated that encapsulation of compounds C1 and C2 in liposomes results in a higher solubility of these compound in aqueous media and a gradual release of C1 under physiological conditions over a period of 7 days. This formulation may solve the problem of poor solubility and difficulty to cross the blood–brain barrier. Further investigation is needed to show proof-of-concept that liposomal formulations of NTDE inhibitors can be used to deliver sufficient amounts of these inhibitors to the brain to modify the dopaminergic pathways, and act as future therapeutics for psychotic disorders.

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References

Afergan, E., Epstein, H., Dahan, R., Koroukhov, N., Rohekar, K., Danenberg, H.D., Golomb, G., 2008. Delivery of serotonin to the brain by monocytes following phagocytosis of liposomes. J. Control. Release 132, 84–90.
Boules, M., Fredrickson, P., Richelson, E., 2006. Bioactive analogs of neurotensin: focus on CNS effects. Peptides 27, 2523–2533.
Caceda, R., Kinkead, B., Nemeroff, C.B., 2006. Neurotensin: role in psychiatric and neurological diseases. Peptides 27, 2385–2404.
Dauch, P., Barelli, H., Vincent, J.P., Checler, F., 1991. Fluorimetric assay of the neurotensin-degrading metalloendopeptidase, endopeptidase 24.16. Biochem. J. 280, 421–426, Pt 2.
de Boer, A.G., Gaillard, P.J., 2007. Drug targeting to the brain. Annu. Rev. Pharmacol. Toxicol. 47, 323–355.
de Boer, A.G., van der Sandt, I.C., Gaillard, P.J., 2003. The role of drug transporters at the blood–brain barrier. Annu. Rev. Pharmacol. Toxicol. 43, 629–656.
De Mei, C., Ramos, M., Iitaka, C., Borrelli, E., 2009. Getting specialized: presynaptic and postsynaptic dopamine D2 receptors. Curr. Opin. Pharmacol. 9, 53–58.
Doulut, S., Dubuc, I., Rodriguez, M., Vecchini, F., Fulcrand, H., Barelli, H., Checler, F., Bourdel, E., Aumelas, A., Lallement, J.C., 1993. Synthesis and analgesic effects of N-[3-[(hydroxyamino) carbonyl]-1-oxo-2(R)-benzylpropyl]-L-isoleucyl-L-leucine, a new potent inhibitor of multiple neurotensin/neuromedin N degrading enzymes. J. Med. Chem. 36, 1369–1379.
Feenstra, R.W., Lange, J.H.M., Pras-Raves, M., L., Kruse, C.G., Van Stuivenberg, H.H., Tuinstra, T., Keizer, H.G., 2003. Neurotensin active 2,3-diaryl-pyparazolidine derivatives. WO/2003/078400, September 25.
Gosk, S., Vermehren, C., Storm, G., Moos, T., 2004. Targeting anti-transferrin receptor antibody (OX26) and OX26-conjugated liposomes to brain capillary endothelial cells using in situ perfusion. J. Cereb. Blood Flow Metab. 24, 1193–1204.
Kinkead, B., Nemeroff, C.B., 2002. Neurotensin: an endogenous antipsychotic? Curr. Opin. Pharmacol. 2, 99–103.
Kitabgi, P., 2006. Inactivation of neurotensin and neuromedin N by Zn metallopeptidases. Peptides 27, 2515–2522.
Kratz, F., 2008. Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. J. Control. Release 132, 171–183.
Lindner, L.H., Hossann, M., 2010. Factors affecting drug release from liposomes. Curr. Opin. Drug Discov. Dev. 13, 111–123.
McCormack, B., Gregoriadis, G., 1994. Entrapment of cyclodextrin–drug complexes into liposomes: potential advantages in drug delivery. J. Drug Target 2, 449–454.
Pan, L., Ho, Q., Tsutsui, K., Takahashi, L., 2001. Comparison of chromatographic and spectroscopic methods used to rank compounds for aqueous solubility. J. Pharm. Sci. 90, 521–529.
Paolino, D., Cosco, D., Racanicchi, L., Trapasso, E., Celia, C., Iannone, M., Puxeddu, E., Costante, G., Filetti, S., Russo, D., Fresta, M., 2010. Gemcitabine-loaded PEGylated unilamellar liposomes vs GEMZAR®: biodistribution, pharmacokinetic features and in vivo antitumor activity. J. Control. Release 144, 144–150.
Pardridge, W.M., 2005. The blood–brain barrier: bottleneck in brain drug development. NeuroRx 2, 3–14.
Pardridge, W.M., 2007. Blood–brain barrier delivery. Drug Discov. Today 12, 54–61.
Peschka, R., Dennehy, C., Szoka Jr., F.C., 1998. A simple in vitro model to study the release kinetics of liposome encapsulated material. J. Control. Release 56, 41–51.
Rouser, G., Fleischer, S., Yamamoto, A., 1970. Two dimensional then layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. Lipids 5, 494–496.
Schnyder, A., Krahenbuhl, S., Drewe, J., Huwyler, J., 2005. Targeting of daunomycin using biotinylated immunoliposomes: pharmacokinetics, tissue distribution and in vitro pharmacological effects. J. Drug Target 13, 325–335.
Seeman, P., 2010. Dopamine D2 receptors as treatment targets in schizophrenia. Clin. Schizophr. Relat. Psychoses 4, 56–73.
Shi, N., Zhang, Y., Zhu, C., Boado, R.J., Pardridge, W.M., 2001. Brain-specific expression of an exogenous gene after i.v. administration. Proc. Natl. Acad. Sci. U. S. A. 98, 12754–12759.
van Rooy, I., Cakir-Tascioglu, S., Hennink, W.E., Storm, G., Schiffelers, R.M., Mastrobattista, E., 2010a. In vivo methods to study uptake of nanoparticles into the brain. Pharm. Res..
van Rooy, I., Mastrobattista, E., Storm, G., Hennink, W.E., Schiffelers, R.M., 2010b. Comparison of five different targeting ligands to enhance accumulation of liposomes into the brain. J. Control. Release.
Vincent, B., Dive, V., Yiotakis, A., Smadja, C., Maldonado, R., Vincent, J.P., Checler, F., 1995. Phosphorus-containing peptides as mixed inhibitors of endopeptidase 3.4.24.15 and 3.4.24.16: effect on neurotensin degradation in vitro and in vivo. Br. J. Pharmacol. 115, 1053–1063.
Wu, J., Liu, Q., Lee, R.J., 2006. A folate receptor-targeted liposomal formulation for paclitaxel. Int. J. Pharm. 316, 148–153.
Xie, Y., Ye, L., Zhang, X., Cui, W., Lou, J., Nagai, T., Hou, X., 2005. Transport of nerve growth factor encapsulated into liposomes across the blood–brain barrier: in vitro and in vivo studies. J. Control. Release 105, 106–119.
Zhang, X., Xie, Y., Jin, Y., Hou, X., Ye, L., Lou, J., 2004. The effect of RMP-7 and its derivative on transporting Evans blue liposomes into the brain. Drug Deliv. 11, 301–309.