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Distribution of an intravenous injectable nimodipine nanosuspension in mice

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Abstract

The distribution of an intravenous injectable nimodipine nanosuspension with mean particle size of both 300 and 650 nm in mice was systemically investigated compared with that of a nimodipine ethanol formulation (Nimotop) and a nanosuspension coated with Tween-80. The results showed that the 650-nm nanoparticles provided significantly higher drug concentrations in the liver, spleen and lungs because of their capture by Kupffer cells in the mononuclear phagocyte system, but lower drug concentrations in the brain compared with Nimotop and smaller nanoparticles. These nanoparticles failed to give increased brain concentrations even when coated with Tween-80. The 300-nm nanoparticles could effectively increase drug concentrations in the brain and remarkably reduce drug concentrations in the liver, spleen and lungs, indicating that the nimodipine nanosuspension may be a promising formulation with no ethanol, but the particle size must be small.

Introduction

Nimodipine is a dihydropyridine calcium-channel blocker used in the treatment of senile dementia and in the reduction of the morbidity and mortality associated with delayed ischaemic deficits in patients with subarachnoid haemorrhage related vasospasm (Vinge et al 1986). However, the clinical usefulness of nimodipine was limited by its poor solubility and high first-pass effect in the liver, leading to low bioavailability after oral administration and poor brain targeting. Now, the clinical formulation for intravenous administration of nimodipine (Nimotop) contains about 40.7% solvent mixture (23.7% v/v ethanol and 17% v/v PEG400) to achieve a sufficient nimodipine concentration in the body and the brain. However, with the presence of alcohol in the formulation, pain and irritation were inevitable, and in some circumstances caused extreme phlebitis once the drug solution effused to the outside of blood vessel.

Drug reformulation as nanosuspensions has received a lot of attention in recent years because they contain only a few excipients to form a pure drug colloidal system without any organic solvent, which could effectively reduce the undesirable side-effects, particularly following intravenous administration. Properties of the nanosuspension such as size and size distribution, dissolution velocity and saturation solubility, and surface hydrophilicity/hydrophobicity influence their behaviour in-vivo. After intravenous administration, nanosuspensions with a small size and high dissolution velocity can dissolve quickly in plasma, otherwise they would be phagocytized by the mononuclear phagocytic system (MPS) and passively target the liver, spleen or lungs. If their dissolution in plasma is prolonged, the nanoparticles would be adsorbed by plasma protein, the qualitative and composition of which is generally recognized as the essential key factor for organ distribution (Müller et al 2001). Some studies showed that nanoparticles with Tween 80 modified on the surface could anchor apolipoprotein E, which plays an important role in the transport of low density lipoprotein into brain. Once the Tween-80 coated nanoparticles are capable of interacting with low density lipoprotein receptors on the brain microvessel endothelial cells, they could be further positively transported into brain (Kreuter 2001; Sun et al 2004).

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This study investigated the distribution of a nimodipine nanosuspension produced by high pressure homogenization before and after being coated with Tween-80; the influence of particle size in the distribution was also studied.

Materials and Methods

Materials

Nimodipine and nitrendipine were purchased from Shandong Xinhua Pharmaceutical Factory, China. Poloxamer 188 was given by BASF, Germany. Sodium deoxycholate and mannitol were purchased from Sinopharm Chemical Reagent Co., Ltd, China. Polysorbate 80 was obtained from Beijing Fengli Jingqin Commerce and Trade Co., Ltd, China. Nimotop was produced by Bayer, Germany. All other reagents were of the highest grade commercially available.

Preparation of nimodipine nanosuspension

The nimodipine nanosuspension was prepared according to Xiong et al (2008). The nimodipine coarse powder was first disintegrated into microparticles using an MC One fluid jet mill (Jetpharma SA, Balerna, Switzerland). Then, the nimodipine microparticles (0.5%) were dispersed in an aqueous surfactant solution containing 0.6% poloxamer 188, 0.4% sodium cholic acid and 4.0% mannitol with magnetic stirring. The pre-mixture obtained was homogenized under a pressure of 1000 bar for 25 cycles in a Niro-Soavi NS1001L homogenizer (ATS Co., Ltd, Italy). After being lyophilized and reconstructed with pure water, the nimodipine nanosuspension was diluted with physiological saline to approximately 0.2 mg mL^{-1} (the same concentration as the clinical nimodipine ethanol solution) for in-vivo testing. Then, 0.5% Tween-80 was added for surface modification. After incubating for 30 min with gentle magnetic stirring, the nanoasuspension was immediately injected intravenously into mice (Kreuter 2001). The particles were also centrifuged at 9000 rev min^{-1} (12 000 × g) for 10 min to achieve smaller nanoparticles.

Characterization of the nimodipine nanosuspension

Particle size analysis was performed by photon correlation spectroscopy using a Nicomp 380/ZLS (PSS; Santa Barbara, CA, USA). The diameters were calculated in volume-weighted Gaussian distribution. Diameter 99% meant that 99% of the particles were below the given size. The polydispersity index indicated the width of a particle size distribution (e.g. 0.0 for a narrow distribution, >0.3 for a very broad distribution) (Peters et al 2000).

Body distribution studies

Animal experiments

Experiments involving mice were approved by the Ethics Committee of Shanghai Institute of Pharmaceutical Industry. Male Kunming mice (18–22 g) were given a single intravenous injection of nimodipine nanosuspension or control ethanol solution (Nimotop) through the tail vein at a dose of 1 mg kg^{-1}. At each predetermined time point (2, 5, 10 and 15 min) four mice were killed by decapitation, and blood, brain, liver, spleen, lungs and kidney were sampled. After centrifugation for plasma, all samples were kept at −20°C until high-performance liquid chromatography (HPLC) analysis.

Analytical procedures

Nimodipine in plasma and tissues was assayed by a HPLC method as described by Zhang et al (2004). A 200-μL plasma sample or weighed tissue homogenate, 200 μL nitrendipine (500 μg mL^{-1} as internal standard) and 200 μL 1 M NaOH were added into a glass tube. After briefly mixing, they were extracted with 2 mL or 5 mL extraction solvent (*n*-hexane/diethyl ether 1:1) by vortex for 10 min. After centrifugation at 3000 rev min^{-1} (4000 × g) for 10 min, the supernatant was transferred to a conical tube and dried under a gentle stream of nitrogen at 40°C. The residue was reconstituted in 50 μL methanol and 25 μL was injected for HPLC analysis. The limit of quantitation measured based on a signal/noise ratio of ≥10 was 4 ng for this assay. Chromatographic separation was achieved at 35°C on a C_{18} analytical column (250 mm × 4.6 mm, 5 μm; Discovery, Bellefont, PA, USA). The detection wavelength was 358 nm. The mobile phase was 0.05 M ammonium acetate/acetonitrile (40:60 v/v) at a flow rate of 1.2 mL min^{-1}.

Data analysis

The area under the concentration–time curve (AUC) was calculated from time zero to the last data point with extrapolated C_0 and without extrapolation to infinity (Kinetica 2000 software). The variance for the $AUC_{0\to t}$ was estimated by Equation 1 (Yuan 1993):

$$SD = \sqrt{\left(\frac{1}{2}(t_1 - t_0)SEM\right)^2 + \sum_{i=2}^{n}\left(\frac{1}{2}(t_i - t_{i-2})SEM_{i-1}\right)^2 + \left(\frac{1}{2}(t_n - t_{n-1})SEM_n\right)^2} \quad (1)$$

$$SEM_i = S_i \Big/ \sqrt{N_i}$$

where S_i is the standard deviation of the concentration at certain time and N_i is the sample number at a certain time.

Overall targeting efficiency (TE) was defined as the AUC of each tissue divided by the sum AUC of all the tissues:

$$TE = \frac{AUC_{0\to 15}}{\sum AUC_{0\to 15}} \times 100\% \quad (2)$$

When considering the different weight of each tissue, the relative mass (RM) could reveal the distribution of nimodipine

in each tissue more precisely than the targeting efficiency, and was calculated as:

$$RM = \frac{C^*W}{\sum C^*W} \times 100\% \quad (3)$$

where C represents the concentration in each tissue, W represents the weight of the tissue and the denominator represents the sum amount of drug exposure to all the sampled tissues. $\overline{RM}$ was the average of the RM at all the time points, which could reflect the state of the drug deposition in every tissue throughout the experiment.

Individual differences between the various formulations in nimodipine concentration in each body part was statistically examined using a non-parametric test: Kruskal–Wallis test (SPSS 13.0 software; SPSS, Chicago, IL, USA). $\overline{RM}$ was statistically analysed using Tukey's honestly significant difference test following one-way analysis of variance (SPSS 13.0). A level of $P < 0.05$ denoted significance in all cases.

Results and Discussion

Characterization of nimodipine nanosuspensions

After being lyophilized and reconstructed, the particle size of the nimodipine nanosuspension produced by continuous high pressure homogenization was about 650 nm, with a polydispersity index of 0.183. Smaller nanoparticles were obtained after centrifugation, giving a particle size of about 300 nm and a polydispersity index of 0.073 (Table 1).

In a previous study (Xiong et al 2008), the saturation solubility of nimodipine raw crystals and nanocrystals (650 nm and 300 nm) were investigated by retrodialysis methods. It was proved that the saturation solubility of nimodipine doubled after entering the nanometre range and further increased from 5.07 $\mu g\,mL^{-1}$ (650 nm) to 5.42 $\mu g\,mL^{-1}$ (300 nm).

In-vivo tests in mice

Distribution of the 650-nm nimodipine nanosuspension

Nimodipine concentrations in the various tissues are shown in Figure 1. Obviously, the 650-nm nanoparticles had remarkably higher concentrations compared with the Nimotop group in organs such as the liver, spleen and lungs because of the active MPS, as they were just within the ideal size range of the Kupffer cells and could be easily captured before they dissolved completely (Müller et al 2001; Rabinow 2005). In plasma, the concentration of the 650-nm nanoparticles was much higher than that of the Nimotop group, especially at the first time point; after 5 min they were quite similar when the nanoparticles were dissolved from the MPS. So, we could ascribe the low concentrations of the 650-nm nanoparticles in the brain to their slow dissolution rate in the plasma. Although the plasma concentrations were high at the first point, the nimodipine molecular concentrations in plasma were very low. Interestingly, after they were dissolved in the plasma 10 min later, they were still capable of providing brain concentrations that were slightly higher than or similar to that of the Nimotop group (10 and 15 min). So, we could conclude that the critical factor for the nanoparticles to provide molecular drug concentrations capable of penetrating into the brain is their dissolution rate in the blood.

Tween-80 failed to change the deposition fate of the nanoparticles: the concentrations in various tissues in mice were the same for the nanoparticles with or without Tween-80 surface modification (see Figure 1). According to the literature, Tween-80 on the surface of nanoparticles may anchor apolipoprotein E and mimic low density lipoprotein for transportation across the blood–brain barrier (Lück et al 1998; Gao & Jiang 2006). However, in this experiment, Tween-80 could neither decrease the tendency for the nanoparticles to be taken up by the MPS, nor increase the brain transportation. We presume that the surface of the nimodipine nanocrystals was too smooth for the Tween 80 to adhere to and so the apolipoprotein E would hardly be anchored.

Distribution of the 300-nm nimodipine nanosuspension

The deposition of the small nanoparticles with a particle size of 300 nm was dramatically different compared with that of the 650-nm nanoparticles, yet they only differed in relation to particle size and had the same formulation components. The concentrations in liver, spleen and lungs were low, and in the plasma the concentrations were the same as that of the Nimotop group. It was confirmed that the small nanoparticles could quickly dissolve in the blood after intravenous injection and could enter a totally molecular state or enter into a very small size range to avoid capture by the MPS. Although, the in-vitro test showed that the saturation solubility of nimodipine only increased from 5.07 $\mu g\,mL^{-1}$ (650 nm) to 5.42 $\mu g\,mL^{-1}$ (300 nm), we hypothesized the difference in dissolution rate in the blood would be much bigger.

The dramatically higher brain concentrations of the 300-nm nanoparticles compared with the Nimotop group were remarkable (Tables 2 and 3). The targeting efficiency of the 300-nm nanoparticles was 52% greater than that of Nimotop, while the $\overline{RM}$ was almost 100% greater. If the drug in the

Table 1 Particle size and distribution of nimodipine nanocrystals used for the tissue distribution test

Gaussian distribution	Reconstructed		9000 rev min^{-1} for 10 min	
	Mean diameter	Width of distribution	Mean diameter	Width of distribution
Volume weighted (nm)	646.3	276.6	296.5	80.4
Diameter 99% (nm)	1334.8		536.6	
Polydispersity index	0.183		0.073	

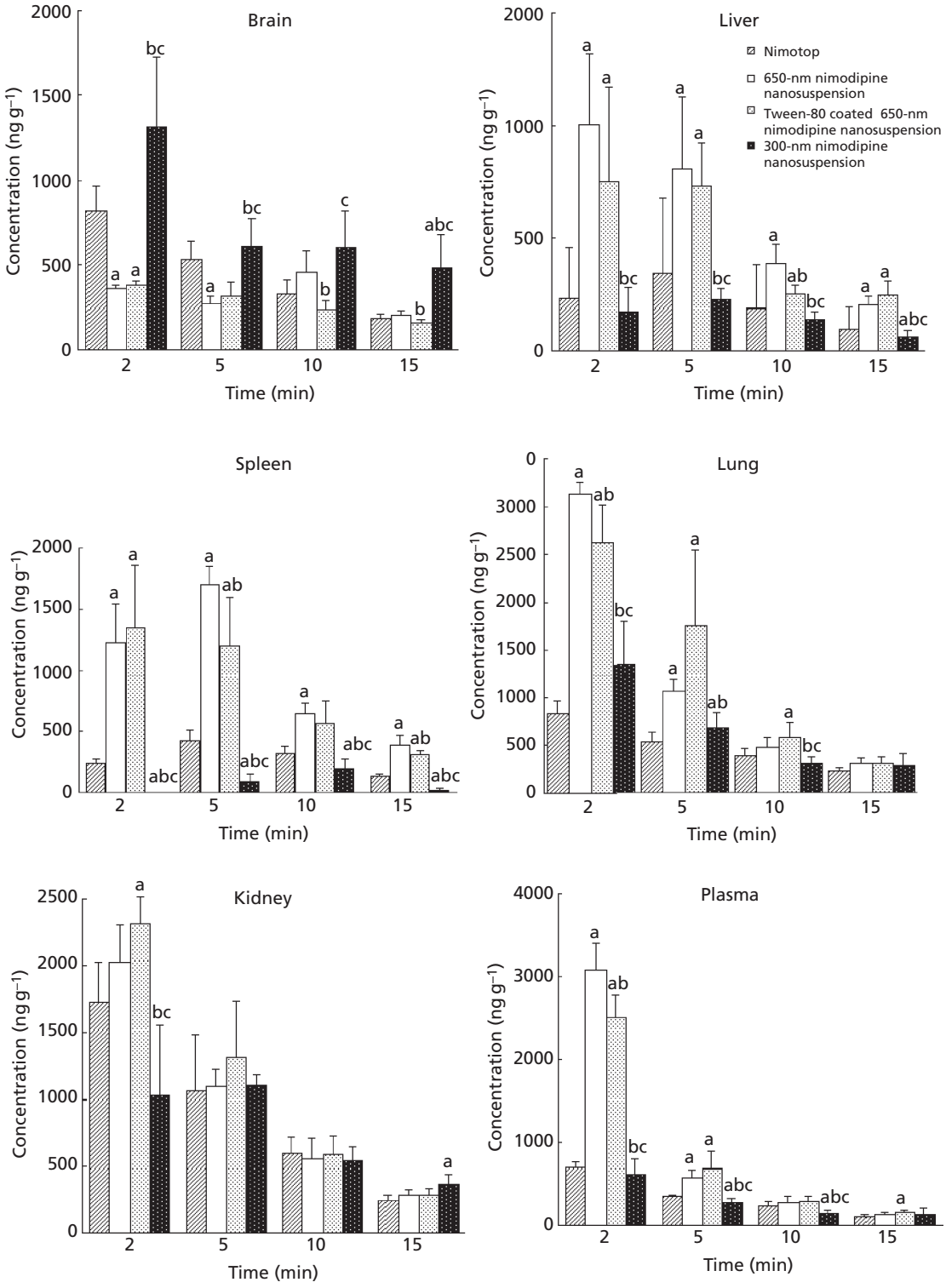

Figure 1 Nimodipine concentrations in tissues after intravenous injection of Nimotop and nimodipine nanosuspensions (n = 4) in mice. [a]$P < 0.05$, significantly different compared with the Nimotop group; [b]$P < 0.05$, significantly different compared with the 650-nm nimodipine nanosuspension group. [c]$P < 0.05$, significantly different compared with the Tween-80 coated 650-nm nimodipine nanosuspension group.

Table 2 The area under the concentration–time curve (AUC) and targeting efficiency in tissues after intravenous injection of Nimotop and nimodipine nanosuspensions in mice

Formulation		Brain	Liver	Spleen	Lung	Kidney	Plasma
Nimotop	AUC (ng h g^{-1})	156.3 ± 2.6	80.7 ± 2.0	91.5 ± 1.7	149.6 ± 2.4	260.5 ± 6.5	121.7 ± 1.5
	Targeting efficiency (%)	18.2	9.4	10.6	17.4	30.3	14.1
650-nm nimodipine nanosuspension	AUC (ng h g^{-1})	125.8 ± 2.6	244.7 ± 5.1	301.5 ± 4.0	314.1 ± 3.0	282.9 ± 4.3	291.7 ± 3.4
	Targeting efficiency (%)	8.1	15.7	19.3	20.1	18.1	18.7
Tween-80 coated 650-nm nimodipine nanosuspension	AUC (ng h g^{-1})	111.3 ± 1.6	180.9 ± 4.5	245.8 ± 7.6	363.1 ± 10.9	316.5 ± 6.3	276.4 ± 3.8
	Targeting efficiency (%)	7.5	12.1	16.5	24.3	21.2	18.5
300-nm nimodipine nanosuspension	AUC (ng h g^{-1})	184.8 ± 9.2	38.9 ± 1.4	20.4 ± 2.9	134.3 ± 14.7	173.0 ± 6.4	70.3 ± 2.4
	Targeting efficiency (%)	27.6	5.8	3.7	23.5	28.4	10.9

Table 3 $\overline{RM}$ of nimodipine in tissues following intravenous injection of Nimotop and nimodipine nanosuspensions (n = 16) in mice

Formulation	Brain	Liver	Spleen	Lung	Kidney	Plasma
Nimotop	14.7 ± 2.3	23.3 ± 7.3	2.4 ± 1.7	6.3 ± 2.9	16.9 ± 3.1	36.4 ± 5.8
650-nm nimodipine nanosuspension	7.3 ± 5.2[a]	35.6 ± 12.0[a]	4.5 ± 3.3[a]	6.4 ± 3.0	9.4 ± 4.5[a]	36.8 ± 12.9
Tween-80 coated 650-nm nimodipine nanosuspension	6.8 ± 2.9[a]	26.8 ± 9.0	5.4 ± 2.8[a]	9.6 ± 2.8	12.6 ± 5.4[a]	38.8 ± 10.6
300-nm nimodipine nanosuspension	28.2 ± 5.9[abc]	14.4 ± 5.0[bc]	1.5 ± 1.3[abc]	9.2 ± 2.2[ab]	17.5 ± 4.0[bc]	29.3 ± 6.5

$\overline{RM}$ was the average of 16 values, comprising 4 time points with four animals at each point. Data are mean ± s.d. (%). [a]$P < 0.05$, significantly different compared with the Nimotop group; [b]$P < 0.05$, significantly different compared with the 650-nm nimodipine nanosuspension group; [c]$P < 0.05$, significantly different compared with the Tween-80 coated 650-nm nimodipine nanosuspension group.

blood of the 300-nm nanoparticles and the Nimotop was in both cases in the molecular state, what led to this difference? We could perhaps attribute the difference to three reasons. (i) There was 23.7% (v/v) alcohol in the formulation of Nimotop. The sudden injection of a large amount of alcohol into plasma would inevitably induce a physiological change in the cerebral vessels, which may have a negative effect on the ability of nimodipine to penetrate into the brain (Zhao et al 2006). (ii) The surfactants in the nanosuspension formulation may be helpful in improving drug penetration. (iii) If the 300-nm nanoparticles were dissolved into a much smaller size range, they could probably adsorb through the capillary walls, and could create a higher concentration gradient that would enhance the transport across the blood–brain barrier. Furthermore, the small nanoparticles may even be endocytosed by the endothelial cells and delivered into the brain.

Conclusion

Although the literature indicates that Tween-80-mediated drugs can cross the blood–brain barrier, we found that large nanocrystals could not be effectively delivered into the brain even after coating with Tween-80. The dissolution rate of the nanoparticles in-vivo played a more important role in influencing not only the distribution fate but also brain drug concentrations.

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