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Koskimäki, Janne

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Absorption, elimination and cerebrospinal fluid concentrations of nimodipine in healthy beagle dogs receiving human intravenous and oral formulation

Janne Koskimäki · Miikka Tarkia ·
Tuula Ahtola-Sättilä · Lasse Saloranta ·
Aki Laakso · Janek Frantzen

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Abstract Nimodipine is an L-type calcium channel blocker and is used to treat vasospasm in patients with subarachnoid hemorrhage. Its putative mechanism of action is relaxation of smooth muscle cells in cerebral arteries. In addition, nimodipine may have pleiotropic effects against vasospasm. Systemic hypotension is an adverse effect when patients are treated with oral or intravenous nimodipine. Intracranial administration of nimodipine formulations may produce higher concentration of nimodipine in the cerebrospinal fluid (CSF) than is possible to achieve orally or intravenously, while resulting in lower incidence of systemic hypotension. The aim of this study was to provide information on plasma and CSF levels of nimodipine in beagle dogs as a comparative data for development of experimental intracranial treatment modalities.

Plasma levels of nimodipine were measured after current 30 and 60 mg single oral dose of nimodipine (Nimotop[®] 30 mg tablets), a single intravenous bolus 0.72 mg/dog of nimodipine (Nimotop[®] 0.2 mg/ml infusion solution) and CSF levels after 60 mg single oral dose of nimodipine. CSF/Plasma concentration ratio of nimodipine after oral administration of 60 mg at 1 h was 0.013 ± 0.0005 . The mean terminal elimination half-life of nimodipine after i.v. bolus dose 0.72 mg was 1.8 h and mean plasma clearance was 40.3 and 3.4 l/h/kg. Absolute bioavailability was 22 %. Maximum plasma concentration and area under the plasma concentration–time curve from time of administration until the last measurable plasma concentration increased in a dose-proportional manner comparing the exposure parameters at oral doses of 30 and 60 mg. Individual variation in the kinetic profile of nimodipine was measured.

J. Koskimäki
Faculty of Medicine, University of Turku, Kiinamylynkatu 4-8,
P.O. Box 52, 20520 Turku, Finland

J. Koskimäki · A. Laakso
Department of Neurosurgery, Helsinki University Central
Hospital, Topeliuksenkatu 5, P.O. Box 266, 00029 Helsinki,
Finland

M. Tarkia
Turku PET Centre, University of Turku and Turku University
Hospital, Kiinamylynkatu 4-8, P.O. Box 52, 20521 Turku,
Finland

T. Ahtola-Sättilä · L. Saloranta
Chemistry and Safety Sciences, R&D, Orion Corporation, Orion
Pharma, Orionintie 1A, P.O. Box 65, 02101 Espoo, Finland

J. Frantzen (✉)
Clinical Neurosciences, Department of Neurosurgery, Turku
University Central Hospital, Hämeentie 11, P.O. Box 52,
20521 Turku, Finland
e-mail: janek.frantzen@tyks.fi

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1 Introduction

As an L-type calcium channel blocker, nimodipine binds specifically with high affinity to L-type voltage-gated calcium channels (VGCC) located on extra- and intracranial blood vessels, neurons, heart and skeletal muscle (Triggle 2006). This results in an inhibition of calcium influx causing relaxation of the smooth muscle cells in a medial layer of cerebral arteries and arterioles (Alborch et al. 1995).

Nimodipine is widely used in humans for treating vasospasm, a severe complication of subarachnoid

hemorrhage (SAH) (Velat et al. 2011). Our understanding of the pathophysiology of vasospasm has developed from pure intracranial vessel spasm to a complex process of early brain injury, secondary injuries and cortical spreading ischemia (Athar and Levine 2012; Caner et al. 2012; Sehba et al. 2012).

Intravenous and oral administration routes of nimodipine are currently generally accepted treatments with clinically proven efficacy against vasospasm (Pickard et al. 1989). Also intra-arterial, intrathecal, intraventricular and intracranial administration routes of nimodipine are described in literature (Barth et al. 2011; Cook et al. 2012; Hänggi et al. 2008). Efficacy of the intracranial treatment is under research and results have been encouraging (Kasuya et al. 2005; Macdonald et al. 2012). Advantages of these new treatment strategies lies in their ability to produce higher nimodipine concentrations in the cerebrospinal fluid (CSF) and lower nimodipine concentrations in the plasma. Higher CSF concentration may be beneficial against vasospasm and secondary injuries, including delayed neuronal apoptosis and microthrombosis (Dreier et al. 2009; Vergouwen et al. 2008, 2011). Additionally, lower plasma concentration of nimodipine can reduce the incidence of the systemic hypotension, which should be avoided in patients suffering from intracranial vasospasm (Cook et al. 2012; Richard 2005).

Because intravenous and oral routes of nimodipine are the current therapeutic approaches to treat vasospasm, new treatment routes have to be compared to the current treatment. Beagle dog is a widely used experimental model among SAH researchers. However, the central and peripheral pharmacokinetics of nimodipine is poorly reported in dogs. This information is crucial because the new intracranial treatment strategies that are under development need to be compared to the existing intravenous and oral treatment strategies in preclinical models. Therefore, we designed this study to investigate pharmacokinetics and CSF concentrations of nimodipine in dogs after peripheral administration, providing a comparative data for development of experimental treatment modalities against vasospasm.

2 Materials and methods

2.1 Animal model

This study was performed using six beagle dogs (age 4 years, weight 12 ± 1.0 kg). The dogs were bred by Harlan-Winkelmann GmbH Hundezucht, Germany. The beagle dog was chosen because the dog may be specie for regulatory safety studies for intracranial treatment routes. During the study, 300 g of diet/day/dog was offered after

the 3-h blood sample on the day of drug administration. Morbidity of the animals was recorded at least twice daily. Clinical signs were noted during the controls, if present.

All procedures were made according to European Community Guidelines for the use of experimental animals and approved by Finnish National Animal Experiment Board (licence ESLH-2009-08809/Ym-23).

2.2 Study treatment

Nimodipine i.v. dose was extrapolated from human therapeutic dose using conversion factor according to Food and drug administration recommendation (FDA 2005). The conversion factor used was 0.54. Orally administrated nimodipine doses were the same (60 mg) and the half (30 mg) dose which are used for humans after aneurysmal SAH. The selected doses were not expected to cause toxic or overt cardiovascular effects in dogs. The formulations used in this study were Nimotop® (Bayer Schering Pharma) 30 mg tablets and Nimotop® (Bayer Schering Pharma) infusion 0.2 mg/ml solution. The washout periods were 6 days at minimum (7 days for dogs 1–3 and 6 for dogs 4–6). Treatment groups are presented in Table 1.

2.3 Blood and cerebrospinal fluid sampling

Blood and cerebrospinal fluid samples were collected for determining nimodipine concentrations. Blood samples were collected from the jugular vein and CSF samples were taken from cisterna magna through atlanto-occipital space. The exact sampling time points were recorded and are presented in Table 1. Anesthesia for CSF sampling was induced using dexmedetomidine 20 µg/kg i.v. and a slow propofol bolus 3 mg/kg i.v.

2.4 Sample handling

Blood samples of approximately 3 ml were taken into pre-cooled K₂-EDTA tubes and kept on ice. Samples were protected from light until centrifugation (1,500 g, 10 min, at +4 °C). 700 µl of the separated plasma was used as primary sample stored in an amber-colored polypropylene tube (Eppendorf® Safe-Lock® microcentrifuge tubes volume 1.5 ml amber) and the rest was stored as a spare sample.

Cerebrospinal fluid samples (~1,500 µl) were let to drop freely from a sterile cerebrospinal fluid needle into a sterile amber-colored polypropylene collection tube (Eppendorf® Safe-Lock® tubes volume 2.0 ml). Cerebrospinal fluid samples were kept protected from light, until the separation of supernatant by centrifugation (1,500 g, 10 min, at +4 °C). The separated CSF was divided into two amber-colored polypropylene tubes. 700 µl was taken

Table 1 Treatments and sampling

Treatment	Animal no	Dosing route	Dose mg/dog	Formulation	Blood sampling time postdose	Cerebrospinal fluid sampling time
1 Nimotop [®] infusion 0.2 mg/ml	1–3	i.v.	0.72 (3.6 ml)	Slow i.v. bolus (in 30 s, with covered syringes due to light sensitivity)	Predose, 10, 20, 30, 45, 60 and 90 min, 2 h, 3 h, 5 h, 7 h and 24 h	–
2 Nimotop [®] 30 mg tablet	1–3	p.o.	30	1 × 30 mg tablet	Predose, 20, 40, 60 and 90 min, 2 h, 3 h, 5 h, 7 h and 24 h	–
3 Nimotop [®] 30 mg tablet	4–6	p.o.	60	2 × 30 mg tablet	1 h	1 h
4 Nimotop [®] 30 mg tablet	4–6	p.o.	60	2 × 30 mg tablet	Predose, 20, 40, 60 and 90 min, 2 h, 3 h, 5 h, 7 h and 24 h	–

to nimodipine analysis any excess CSF was stored as a spare sample.

Since nimodipine is very photosensitive, all samples were handled so that exposure to light was minimized. The samples were stored frozen at -80°C until shipping to the laboratory.

2.5 Nimodipine analysis

Nimodipine concentrations in dog plasma and CSF samples were determined by qualified liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods at CRST, Turku, Finland. Sample preparation was performed by liquid–liquid extraction. The extracts were analyzed using reverse-phase chromatography followed by mass spectrometry. The lower limit of quantification (LLOQ) for the plasma method 238 v04 (Rinne and Scheinin 2012a) and the CSF method 240 v03 (Rinne and Scheinin 2012b) was 0.01 ng/ml.

2.6 Pharmacokinetic analysis

The pharmacokinetics of nimodipine was characterized using non-compartmental methods for oral administration and two-compartmental model for i.v. administration. Pharmacokinetic analysis was performed using the WinNonlin 5.0.1 program (Pharsight Corporation, Mountain View, CA, USA). The apparent maximum concentration of nimodipine in plasma (C_{max}) and apparent time to maximum plasma concentration of nimodipine in plasma (t_{max}) were obtained directly from the observed data. The areas under the nimodipine plasma concentration–time curves (AUC) were estimated using the trapezoidal method. The parameters estimated

in the two-compartmental analysis included systemic clearance (Cl), steady-state volume of distribution (V_{ss}), distribution half-life ($t_{1/2\alpha}$) and elimination half-life ($t_{1/2\beta}$).

3 Results

Individual kinetic profiles of nimodipine in plasma after i.v. administration are presented in Fig. 1 and after oral administration in Figs. 2 and 3. The individual and mean $\pm$ SD pharmacokinetic parameters for nimodipine in dog plasma after a single intravenous dose are presented in Table 2 and after oral administration in Tables 3. During nimodipine treatment, any adverse effects were not observed in dogs.

The mean elimination half-life of nimodipine after i.v. bolus dose 0.72 mg was 1.8 h and mean plasma clearance was 40.3 l/h and 3.4 l/h/kg. After oral administration of 60 mg nimodipine the mean elimination half-life was 0.89 h. The absolute bioavailability (calculated for the animals dosed intravenously and orally) was 22 %. Dose proportionality was evaluated by comparing the exposure parameters C_{max} and $\text{AUC}_{0-24\text{ h}}$ at oral doses of 30 mg and 60 mg. C_{max} and $\text{AUC}_{0-24\text{ h}}$ increased in a dose-proportional manner. The same individuals were not used in the groups tested at oral doses of 30 and 60 mg. Indeed, individual variation in the kinetic profile of nimodipine was measured.

At 1 h after oral administration, nimodipine can be measured in the CSF in concentrations of about 1 % of the measured plasma concentrations. Plasma and CSF concentrations of nimodipine after oral dosing of 60 mg are presented in Table 4.

Fig. 1 Plasma concentration profiles after a single i.v., bolus dose of 0.72 mg nimodipine. Logarithmic scale. The last time points of dogs no. 1 and 2 are not shown since nimodipine concentration was under LLOQ (0.01 ng/ml)

Fig. 2 Plasma concentration profiles after oral administration of 30 mg nimodipine. Logarithmic scale

Fig. 3 Plasma concentration profiles after oral administration of 60 mg nimodipine. Logarithmic scale

Table 2 Mean pharmacokinetic parameters of nimodipine after i.v. bolus dosing of 0.72 mg

Animal no	AUC (h ng/ml)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	Cl (l/h)	V _{ss} (l)
1	22.7	11	1.4	31.7	37.6
2	13.3	9	1.6	54.0	98.8
3	20.4	10	2.5	35.3	58.9
Mean	18.8	10	1.8	40.3	65.1
SD	4.9	0.02	0.6	12.0	31.1

Table 3 Mean pharmacokinetic parameters of nimodipine after oral dosing of 30 or 60 mg

	Dose (mg)	Animal no	C _{max} (ng/ml)	t _{max} (h)	AUC _{24 h} (h ng/ml)
	30	1	46.8	0.67	104.9
	30	2	212.4	0.67	342.0
	30	3	50.7	1.00	123.3
Mean			103.3	0.78	190.0
SD			94.5	0.19	131.9
	60	4	238.8	1.00	486.8
	60	5	81.2	1.00	273.2
	60	6	345.0	0.67	569.9
Mean			221.7	0.89	443.3
SD			132.8	0.19	153.1

Table 4 Plasma and CSF concentrations of nimodipine at 1 h after a single oral dose of 60 mg nimodipine

Animal no	Conc. (ng/ml)		CSF/Plasma ratio
	Plasma	CSF	
–			
4	102	1.37	0.0134
5	54.6	0.68	0.0125
6	73.4	0.97	0.0132

4 Discussion

This study report provided new data on pharmacokinetics and CSF concentrations of nimodipine in beagle dogs. New treatment routes of nimodipine are currently under development, and intracranial implants or gel seem utilitarian (Macdonald et al. 2012). New treatment strategies need to be compared to standard treatments in the experimental settings before continuing to human studies. Indeed, intravenous and oral routes of nimodipine are the current therapeutic approaches to treat vasospasm. The relative ineffectiveness of oral and i.v. nimodipine treatment against vasospasm has led to a development of alternative administration routes of calcium channel blockers. Intracranial, intrathecal, intraventricular and intra-arterial

routes are described in the literature (Barth et al. 2011; Cook et al. 2012; Hänggi et al. 2008). Although the results from intracranial administration of calcium channel blockers have been promising, comparative data is needed to develop new formulations and therapy designs in the experimental studies to meet the further aim of drug development, human testing (Kasuya et al. 2005).

According to Cook et al. (2012), injection of sustained release poly D,L-lactide coglycolide (PLGA) nimodipine microparticles in the cisterna magna of dogs results in markedly higher nimodipine concentrations in CSF than those achieved after systemic nimodipine administration in our present study, while producing significantly lower nimodipine plasma levels. According to our data, nimodipine CSF/plasma ratio in dogs is about 0.013, whereas, in humans, it has been reported to be only about 0.0026 (Rämsch et al. 1985). The reason for higher CSF/plasma ratio in dogs compared to humans may be because we used the human dose (60 mg) when measuring the concentration of the nimodipine in CSF and plasma. However, the presented study results are in line with the current knowledge of pharmacokinetics of nimodipine and resemble the kinetics seen in humans as expected. A rapid increase in the concentration of nimodipine is seen after p.o. administration that is followed by a steep decrease within a few hours. Thus, oral administration of nimodipine requires multiple administration times per day to achieve a sufficient concentration. Intracranial administration of a sustained release nimodipine formulation challenges the current six times per day dosing for just one dose administrated during surgery.

Future experimental and clinical studies reveal how treatment strategies of SAH develop and which new treatment modalities achieve a place in daily practise of vascular neurosurgery. Indeed, new better approaches to treat vasospasm are needed. Our research provided basic data for pharmacokinetics of nimodipine in dogs that serves as a comparative data for regulatory safety evaluation of experimental studies and evaluating new administration routes of nimodipine.

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