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Nicardipine, a calcium antagonist, does not aggravate intracerebral haemorrhage in an intracerebral haemorrhage model in rats

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Abstract

Despite controversy over their safety in patients with intracerebral haemorrhage, calcium antagonists are widely used in the treatment of hypertensive emergencies. Here, we investigated the effects of nicardipine on haematoma size and neurological deficit in a rat model of collagenase-induced intracerebral haemorrhage. Injection of collagenase (0.014 U) into the striatum induced haematoma ($19.9 \pm 3.4\,mm^3$) in the striatum and brain oedema. Drugs were infused from 30 min after collagenase injection for 3 h under conscious conditions. Nicardipine intravenously at 0.1, 1 and 10 $\mu g\,kg^{-1}\,min^{-1}$ affected neither haematoma size nor the degree of brain oedema. Nicardipine at these doses provided a stable and dose-dependent decrease in mean blood pressure of 6%, 13% and 33%, respectively, with an increase in heart rate that was apparently caused reflexively. Further, nicardipine did not aggravate the neurological deficits in these intracerebral haemorrhage rats, primarily forearm flexion behaviour on suspension by the tail and circling behaviour. These results indicate that nicardipine infusion stably decreased blood pressure without affecting intracerebral haemorrhage in an intracerebral haemorrhage model in rats.

Introduction

Several models of cerebral haemorrhage have been developed (Bullock et al 1984; Rosenberg et al 1990; Wagner et al 1996). Among them, intracerebral haemorrhage has been studied experimentally using an injection of autologous blood or implantation of inflatable balloons into the caudate nucleus (Ropper & Zervas 1982; Bullock et al 1984; Kaufman et al 1985), because the most common site of intracerebral haemorrhage in humans is the basal ganglia and thalamus (Garcia & Anderson 1991). To achieve a more reproducible haematoma, a rat model in which collagenase was injected into the striatum was developed. The collagenase injection resulted in disruption of the basal lamina of cerebral capillaries, bleeding in the brain parenchyma beginning 30 min after injection and the formation of a haematoma at 4 h (Del Bigio et al 1996). Data from these studies indicate that this intracerebral haemorrhage and subsequent brain damage might involve a space-occupying effect, brain oedema, ischaemia, neurotoxicity or any combination of these (Jenkins et al 1989; Lee et al 1996; Matz et al 1997; Peng et al 1997), suggesting that it relatively closely represents the clinical features of primary intracerebral haemorrhage in humans. As part of our studies on drugs which may affect intracerebral haemorrhage, we previously investigated the relationship between bleeding amount and haematoma size in haemorrhage induced by various dosages of collagenase, and established a sensitive intracerebral haemorrhage rat model for the detection of exacerbatory effects on intracerebral haemorrhage (Terai et al 2003a, b). The data indicated that an optimum model would involve a small intracerebral haemorrhage induced with a low dose of collagenase.

There is a subset of stroke patients (approximately 11–32%) who are known to have a primary intracerebral haemorrhage (Anderson et al 1994; Burchfiel et al 1994; Lo et al 1994). Mortality in the first month after primary intracerebral haemorrhage is 32–55% (Brown et al 1996) and recovery in survivors is poor, most being left with a considerable functional deficit (Daverat et al 1991; Fogelholm et al 1992). Much effort has been

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directed toward treatment for intracerebral haemorrhage (Poungvarin et al 1987; Yu et al 1992; Lapchak & Araujo 2003). Current management of intracerebral haemorrhage includes the control of systemic hypertension and treatment or prevention of raised intracranial pressure (Rincon & Mayer 2004). Although calcium antagonists are used for immediate antihypertensive therapy in patients with intracerebral haemorrhage, their safety in these patients is controversial owing to the possible risk of increased bleeding and intracranial pressure (Hirose et al 1991; Kario et al 1998; Nishiyama et al 2000). The calcium antagonist nimodipine increased cerebral blood flow without inducing or exacerbating oedema or intracerebral pressure in a rat model induced by injection of autologous arterial blood into the caudate nucleus (Sinar et al 1988).

The calcium antagonist nicardipine is widely used as a parenteral antihypertensive drug in patients with hypertensive emergencies. Infusion of nicardipine decreases blood pressure by 25% in patients with acute cerebral haemorrhage (Nishiyama et al 2000). It also produces potent cerebral vasodilatation and increased carotid blood flow in anaesthetized dogs (Takenaka & Handa 1979) and in patients with subarachnoid haemorrhage (Abe et al 1994). Increase in cerebral blood flow could aggravate the intracerebral haemorrhage. However, it is unknown whether nicardipine aggravates intracerebral haemorrhage, subsequent brain oedema or neurological deficits. Here, we investigated the effects of nicardipine on intracerebral haemorrhage and neurological deficits in the rat intracerebral haemorrhagic model.

Materials and Methods

Preparation of the intracerebral haemorrhage model

All experiments were approved by the Animal Ethics Committee of Yamanouchi Pharmaceutical Co. Ltd and complied with the regulations of the committee. These experiments were performed in Panapharm Laboratories Co. Ltd (Kumamoto, Japan), according to the protocols prepared by Yamanouchi Pharmaceutical Co. Ltd (Tokyo, Japan). The intracerebral haemorrhage model was prepared using male Sprague-Dawley rats, 309–320 g (Charles River Japan, Yokohama, Japan). The rats were anaesthetized with 2% isoflurane delivered in a gas mixture of 70% nitrous oxide and 30% oxygen. The jugular vein and carotid artery were catheterized before haemorrhage to allow the intravenous infusion of drugs and the monitoring of arterial blood pressure and heart rate, respectively, using polyethylene tube 50 (PE-50) inserted into the respective vessel and externalized through the dorsal neck.

Haemorrhage was induced as previously described (Terai et al 2003 a, b). Briefly, the head was placed in a stereotaxic frame (David Kopf Instruments, CA). The scalp was then incised through the midline and a hole was drilled through the skull. A 30-gauge needle attached to a 50-μL Hamilton microsyringe was inserted into the striatum 3 mm lateral to the midline and 0.2 mm anterior to the coronal suture of the Bregma to a depth of 6.0 mm below the surface of the skull, then withdrawn 0.3 mm. Collagenase (10 U mL^{-1}, type IV, C-5138; Sigma Chemical Co, MO) dissolved in saline was infused through the needle at a dosage of 0.014 U in 1.4 μL for 7 min (0.2 μL min^{-1}) using a microsyringe pump (KDS-100; LMS, Tokyo, Japan). After infusion, the needle was left in place for 1 min, and then slowly removed over a 1-min period. The bone hole was sealed with Spongel (Yamanouchi Pharmaceutical Co., Tokyo, Japan) and a cyanoacrylate adhesive (Alonalpha A; Toagousei Co., Tokyo, Japan). The scalp wound was sutured and the rat was placed in a warm cage with free access to food and water. When the intracerebral haemorrhage procedure was finished, anaesthesia was discontinued and the rat was allowed to regain consciousness.

Measurement of mean blood pressure and heart rate

After awakening from anaesthesia, the carotid artery catheter was connected to a pressure transducer (P23XL; Gould Electronics, OH) and blood pressure data were transmitted to a pressure processor signal conditioner (Gould Electronics) and recorded on a thermal array recorder (RS3400; Gould Electronics). Measurement was conducted from before the start until the completion of administration under conscious and unrestrained conditions. Evaluation was made using mean blood pressure and heart rate obtained before and at 0.25, 0.5, 1, 2 and 3 h after the start of drug administration.

Drug administration

Nicardipine hydrochloride injection is sold and promoted in several countries, including Japan, by Yamanouchi Pharmaceutical Co. Ltd, as an antihypertensive drug for use in patients with hypertensive emergencies. Nicardipine hydrochloride (Lot no. 51K1532; Sigma, MO) was obtained commercially. The administration schedule is described in Figure 1. Intravenous infusion of nicardipine hydrochloride and saline for control were commenced 30 min after the end of collagenase injection at a rate of 5 mL kg^{-1} h^{-1} for 3 h. A dose of 10 μg kg^{-1} min^{-1} was used as a high dose on the basis of preliminary data, which showed a decrease in mean blood pressure of more than 20% at this level. At the end of infusion, the rats were individually housed in regular cages for animal care.

Figure 1 Experimental protocol. Intravenous infusion of drug for 3 h (black bar) was commenced at 30 min after 7-min injection of collagenase at 10 U mL^{-1} (1.4 μL, 0.014 U, gray bar).

Pathological analysis

Twenty-four hours after collagenase injection, the brain was quickly removed, immersed in ice-cold saline for 1 min and set on a tissue chopper (McIlwain Laboratory Engineering Co., Surrey, UK). Coronal sections of 1-mm thickness from the anterior to posterior poles of the cerebral cortex were mounted on glass slides and 10 brain sections transversing the whole striatum were photographed in colour. The images were analysed using Adobe Photoshop 3.0J (Adobe System, USA), with the area of haematoma (mm^2) in each section determined from the red-coloured area in the striatum. Haematoma volume (mm^3) was calculated from the haematoma area (mm^2) in each 1-mm coronal section. Brain oedema volume was calculated using the following formula:

$$\text{Oedema (\%)} = \text{haematoma hemisphere volume/non-haematoma hemisphere volume} \times 100 \quad (1)$$

Observation and scoring of neurological deficits

Neurological deficits were evaluated according to the method of Bederson et al (1986) with each rat housed individually in a regular cage for animal care at 24 h after collagenase injection. The observation was carried out in a blind manner to avoid subjective factors. The scoring criteria were 1 point each for forearm flexion and rotation behaviour when suspended by the tail, and circling behaviour. Minimum and maximum total scores were 0, representing severe deficit, and 3 for normal rats, respectively.

Statistical analysis

Values for mean blood pressure, heart rate, haematoma volume and oedema were expressed as the mean ± s.e.m. of 8 rats in each group. Statistical differences in mean blood pressure and heart rate between the drug and control groups were analysed using two-way repeated measures analysis of variance followed by Dunnett's test. Statistical differences in haematoma volume and oedema between the drug and control groups were analysed using Dunnett's test. Non-parametric neurological deficit scores were expressed as the median, while statistical differences between the drug and control groups were analysed using Steel's test. $P < 0.05$ was considered statistically significant. All data analyses were performed using the SAS statistical software (SAS Institute, Cary, NC).

Results

Haematoma volume and oedema

Intrastriatal injection of collagenase (0.014 U) induced clear intracerebral haemorrhage, which was restricted to the striatum and of an appropriate size ($19.9 \pm 3.4\,mm^3$, Figure 2). The volume of the haematoma hemisphere was greater than that of the non-haematoma hemisphere, demonstrating the presence of oedema. As shown in Table 1, nicardipine (0.1, 1 and $10\,\mu g\,kg^{-1}\,min^{-1}$, i.v.) had no effect on haematoma volume ($24.0 \pm 3.7\,mm^3$, $25.5 \pm 4.7\,mm^3$ and $20.6 \pm 4.3\,mm^3$, respectively), and no significant difference was seen in the degree of brain oedema among the groups.

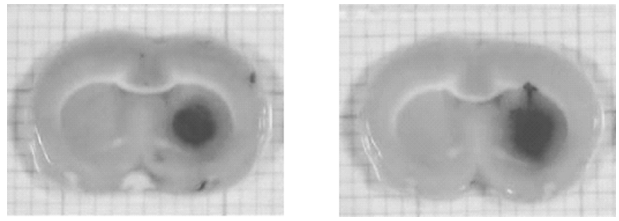

Figure 2 A representative haematoma induced by collagenase injection in rats. The haematoma was induced by 0.014 U of collagenase injected into the striatum and is restricted to the striatum and of an appropriate size. Nicardipine was intravenously infused for 3 h. The brain was removed 24 h after drug administration.

Table 1 Effect of nicardipine on haematoma volume and oedema in the intracerebral haemorrhage rat model

Drug	Dose ($\mu g\,kg^{-1}\,min^{-1}$)	n	Haematoma (mm^3)	Oedema (%)
Control	Saline	8	19.9 ± 3.4	105.5 ± 1.4
Nicardipine	0.1	8	24.0 ± 3.7	103.2 ± 1.6
Nicardipine	1	8	25.5 ± 4.7	104.6 ± 1.6
Nicardipine	10	8	20.6 ± 4.3	105.6 ± 0.9

Collagenase (0.014 U) was injected into the striatum to induce haematoma 0.5 h before drug infusion. Drugs were intravenously infused for 3 h. The brain was removed 24 h after drug administration. Haematoma volume was calculated by integration of the haematoma area in 1-mm-thick brain slices. Brain oedema was calculated by the following formula: oedema (%) = (haematoma hemisphere volume/non-haematoma hemisphere volume) × 100. Each value represents the mean ± s.e.m. No significant difference was shown in haematoma volume and oedema compared with the control group (Dunnett's test).

Mean blood pressure and heart beat

The effect of nicardipine on mean blood pressure in the intracerebral haemorrhage rats is shown in Figure 3. Mean blood pressure before saline infusion in the control group was 107 ± 1 mmHg, and was maintained at 101–109 mmHg during saline infusion, giving a percentage change from the pre-infusion value of −6% to 2%. Mean blood pressure before administration in the nicardipine-treated groups was 105–110 mmHg. Nicardipine at 0.1, 1 and $10\,\mu g\,kg^{-1}\,min^{-1}$ intravenously induced a stable and dose-dependent decrease in pressure during administration to 99–108, 92–103 and 73–77 mmHg, respectively, giving a percentage change from the pre-infusion value of −6% to 3%, −13% to −2% and −33% to −30%,

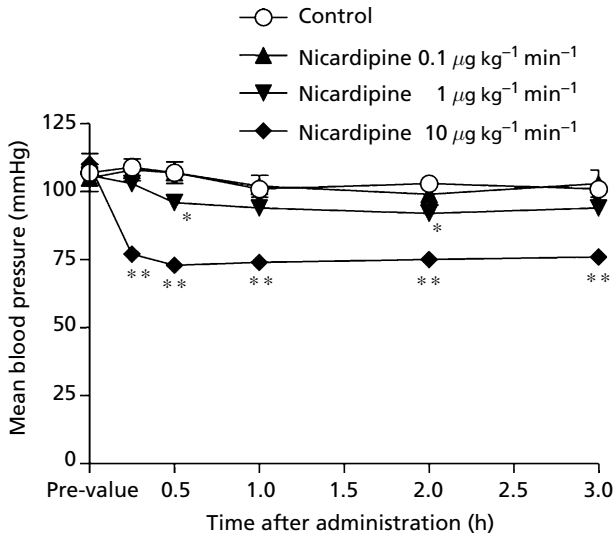

Figure 3 Effect of nicardipine on mean blood pressure in the intracerebral haemorrhage rat model. Collagenase (0.014 U) was injected into the striatum to induce haematoma 0.5 h before drug infusion. Drugs were intravenously infused for 3 h. Each value represents the mean ± s.e.m. of 8 rats. $*P < 0.05$, $**P < 0.01$ compared with the control group (Dunnett's test).

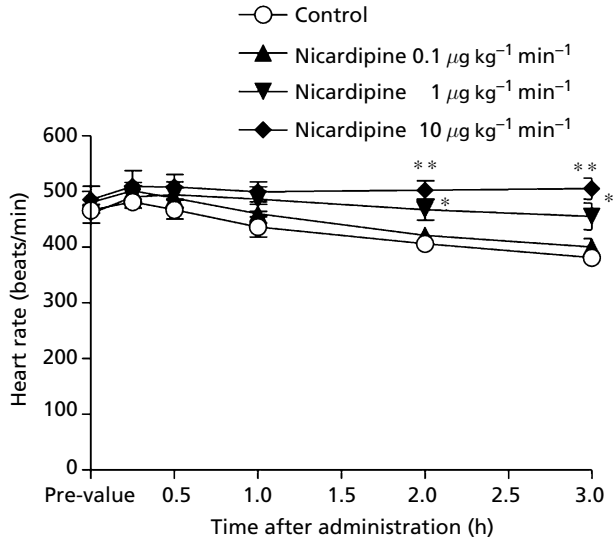

Figure 4 Effect of nicardipine on heart rate in the intracerebral haemorrhage rat model. Collagenase (0.014 U) was injected into the striatum to induce haematoma 0.5 h before drug infusion. Drugs were intravenously infused for 3 h. Each value represents the mean ± s.e.m. of 8 rats. $*P < 0.05$, $**P < 0.01$ compared with the control group (Dunnett's test).

respectively. The effect of nicardipine on heart rate in the cerebral haemorrhage rats is shown in Figure 4. Heart rate before saline infusion in the control group was 466 ± 14 beats/min, and was maintained at 381–481 beats/min during infusion, giving a percentage change from the pre-infusion value of −18% to 3%. Heart rate before administration in the nicardipine-treated groups was 460–485 beats/min. Nicardipine at 0.1, 1 and 10 $\mu g\,kg^{-1}\,min^{-1}$ intravenously induced a dose-dependent increase in heart rate during administration to 400–501, 455–494 and 499–509 beats/min, respectively, giving a percentage change from the pre-infusion value of −17% to 4%, −1% to 8% and 4% to 5%, respectively.

Neurological deficits

The effect of nicardipine on neurological deficits in the cerebral haemorrhage rats as assessed 24 h after collagenase injection according to the method of Bederson et al (1986) is shown in Table 2. The main deficits were forearm flexion behaviour on suspension by the tail and circling behaviour. The median of total deficit scores in the control group was 2. Nicardipine at 0.1, 1 and 10 $\mu g\,kg^{-1}\,min^{-1}$ intravenously had no effect on these scores, with median values of 2, 2 and 2, respectively.

Discussion

The calcium antagonist nicardipine increased carotid blood flow in anaesthetized dogs (Takenaka & Handa 1979) and in patients with subarachnoid haemorrhage (Abe et al 1994). Here, because any increase may affect the development of cerebral haemorrhage and brain oedema, we evaluated the effects of nicardipine on haematoma size and oedema in a rat cerebral haemorrhage model. Our results showed that nicardipine did not influence haematoma volume or brain oedema at doses of 1–10 $\mu g\,kg^{-1}\,min^{-1}$ intravenously, at which it showed an antihypertensive effect. These findings suggest that nicardipine has no aggravating effects on intracerebral haemorrhage. In this study, intrastriatal injection of a low dose of collagenase (0.014 U) induced a relatively small intracerebral haemorrhage (19.9 mm^3), this size being consistent with our previous data (16.3–25.1 mm^3) (Terai et al 2003a). A smaller haematoma is necessary for the detection of any aggravating effect (Terai et al 2003b).

Although our data for nicardipine were in accordance with those previously reported for calcium and serotonin antagonists (Elger et al 1994; Rosenberg & Navratil 1994), these drugs have not been investigated for their effects on neurological deficits and blood pressure in this cerebral haemorrhage model. We examined the effects of nicardipine on neurological deficits 1 day after collagenase injection in the same intracerebral haemorrhage rats. Results showed nicardipine did not influence neurological deficit. Acute neurological deficits in this model can result from a number of causes, including direct tissue destruction, the space-occupying effect of the haematoma and potential ischaemic damage to adjacent tissue, brain oedema, or any combination of these (Del Bigio et al 1996, 1999). They are most severe 1–3 days after haemorrhage and almost completely resolved by one month (Jenkins et al 1989; Del Bigio et al 1996; Lee et al 1996; Matz et al 1997). We previously demonstrated closely similar results

Table 2 Effect of nicardipine on neurological deficits in the intracerebral haemorrhage rat model

Drug	Dose ($\mu g\,kg^{-1}\,min^{-1}$)	n	Median score	No. of rats scored by neurological deficit			
				Score 0	Score 1	Score 2	Score 3
Control	Saline	8	2	0	1	7	0
Nicardipine	0.1	8	2	0	0	8	0
Nicardipine	1	8	2	1	0	7	0
Nicardipine	10	8	2	0	1	7	0

Collagenase (0.014 U) was injected into the striatum to induce haematoma 0.5 h before drug infusion. Drugs were intravenously infused for 3 h. Neurological deficits were measured 24 h after drug administration. No significant difference was shown in neurological score compared with the control group (Steel's test).

in this model (Terai et al 2003b). We consider the finding that nicardipine did not exacerbate the neurological deficits 1 day after intracerebral haemorrhage induction in this model as further evidence that it has no effect on haematoma development or generalized oedema.

Approximately half of all clinical cases of intracerebral haemorrhage are associated with hypertension, and the early use of antihypertensive drugs is recommended in patients with hypertensive emergencies (Emergency Cardiac Care Committee and Subcommittees 1992). In this study, nicardipine infusion ($0.1–10\,\mu g\,kg^{-1}\,min^{-1}$, i.v.) decreased mean blood pressure in a rat intracerebral haemorrhage model in a stable and dose-dependent manner. The degree of decrease, namely 13% and 33% at 1 and $10\,\mu g\,kg^{-1}\,min^{-1}$ intravenously, respectively, was consistent with those in previous studies in which nicardipine by intravenous infusion at $2.5–4.5\,\mu g\,kg^{-1}\,min^{-1}$decreased mean blood pressure by 20% in conscious spontaneously hypertensive rats (Calhoun et al 2001) and nicardipine at $0.6–1.1\,\mu g\,kg^{-1}\,min^{-1}$decreased it by 20–30% in patients with acute cerebral haemorrhage (Nishiyama et al 2000). We consider that the hypotensive effects of nicardipine were sufficient to examine its effects on intracerebral haemorrhage.

Our results from the rat intracerebral haemorrhage model are not consistent with the contra-indication against nicardipine injection in patients with suspected incomplete haemostasis following intracranial haemorrhage because of the risk that the haemorrhage may be exacerbated. We did not investigate the effects of nicardipine on ischaemic damage to adjacent tissue. While nicardipine has a neuroprotective effect at high doses in a cerebral ischaemia model (Takakura et al 1991; Kittaka et al 1997), there is a report showing it to have no effect on cerebral infarction (Miyazaki et al 1999). However, there is no report showing that nicardipine aggravates cerebral infarction. Because nicardipine did not ameliorate the neurological deficits, it may have no neuroprotective effect on ischaemic damage surrounding haemorrhage in an intracerebral haemorrhage model. Further study should be done to clarify the safety of nicardipine infusion in these patients.

Conclusions

Calcium antagonists have been used for immediate antihypertensive therapy in patients with intracerebral haemorrhage, but their use in patients with intracerebral haemorrhage is controversial owing to the possible risk of increased bleeding and intracranial pressure due to increased cerebral blood flow. We demonstrate here that nicardipine infusion produced a stable and dose-dependent decrease in mean blood pressure with no effect on haematoma volume or brain oedema in a rat intracerebral haemorrhage model.

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