Effects of propofol on lactate accumulation and oedema formation in focal cerebral ischaemia in hyperglycaemic rats

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Background. In cerebral ischaemia, hyperglycaemia brings about severe lactate accumulation and neuronal damage when compared with normoglycaemia. Propofol has been known to suppress glucose metabolism in the brain and possess neuroprotective properties in cerebral ischaemia. Therefore, in this study we examined if propofol could attenuate lactate accumulation and neuronal damage in cerebral ischaemia under hyperglycaemic conditions.

Methods. Ten male wistar rats were divided into two experimental groups: low-dose (~12 mg kg^{-1} h^{-1}) and high-dose (~60 mg kg^{-1} h^{-1}) propofol groups (n=5 for each). Following injection of 2 g kg^{-1} glucose intraperitoneally, the middle cerebral artery was occluded for 1 h, and then reperfused for the following 2 h. Lactate accumulation and oedema formation were estimated consecutively using nuclear magnetic resonance (NMR) techniques.

Results. Lactate accumulation and oedema formation increased continuously during ischaemia and reperfusion in the low-dose propofol group, which was attenuated in the high-dose propofol group. Lactate/NAA (*N*-acetylaspartate) ratio (as an index of lactate accumulation) 60 and 120 min after reperfusion were 2.67 and 3.26 in low-dose group and 0.30 and 0.10 in high-dose group. For NMR images the number of pixels with a low average diffusion coefficient (an index of the oedema formation), 60 and 120 min after reperfusion were 250.0 and 317.8 in low-dose group, and 16.0 and 12.4 in high-dose group.

Conclusion. High-dose propofol attenuated lactate accumulation and oedema formation in cerebral ischaemia in hyperglycaemic rats.

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Pre-ischaemic hyperglycaemia aggravates brain damage following ischaemia.[1–3] Hyperglycaemia significantly worsens both intracellular brain acidosis and mitochondrial function in the ischaemic penumbra.[4] Hyperglycaemia increases the magnitude and topographic extent of tissue lactic acidosis and infarct size following transient cerebral ischaemia.[5] In a previous study, we showed the extent of lactate accumulation and oedema formation in transient focal cerebral ischaemia was more severe in rats under hyperglycaemic conditions than those under normoglycaemic conditions using nuclear magnetic resonance (NMR).[6] Further, it was demonstrated that isoflurane did not attenuate the adverse effects of hyperglycaemia; nevertheless, isoflurane was reported to improve neurological outcome after incomplete cerebral ischaemia in rats.[7]

Propofol, an i.v. anaesthetic, was reported to possess neuroprotective properties,[8–10] which are greater than isoflurane in cerebral ischaemia reperfusion injury.[10] Furthermore, propofol was shown to suppress cerebral glucose utilization[11 12] more effectively than isoflurane.[13] Therefore, propofol may attenuate ischaemia-induced neuronal damage aggravated by hyperglycaemia.

In the present study, the effects of propofol on the accumulation of lactate and the formation of oedema in the brain during ischaemia and reperfusion were examined in hyperglycaemic rats, using NMR technique.

Material and methods

Animal preparation

The study was approved by the Animal Research Committee of Kyoto University, Faculty of Medicine. Experiments were performed on 10 adult spontaneously hypertensive rats (SHR) weighing 310–360 g and aged 12–15 weeks. Rats were allowed free access to water and laboratory chow before experimentation. Each rat was anaesthetized with 2% halothane in 50% oxygen and balanced nitrogen through a facemask. A remote controlled rat intraluminal suture middle cerebral artery occlusion (MCAO) model was prepared as described by Röther and colleagues.[14] The occluding device was prepared with a 4-0 monofilament nylon suture 5-cm long, the tip of which was rounded by heating, and glued to the end of a 2-0 monofilament nylon suture 40 cm in length. The tip of the occluding device was introduced into the left extracranial internal carotid artery through a polyethylene tube sheath inserted and fixed into the left common carotid artery. Catheters were inserted into the right femoral artery and vein for continuous arterial pressure measurement, blood sampling, and drug administration. An additional polyethylene tube was inserted into the abdominal cavity for administration of glucose solution. Following completion of surgery, halothane was discontinued, and an i.v. infusion of propofol was begun. Rats were fixed in an acryl cradle in the supine position and placed in the NMR device. The animals were assigned to one of two experimental groups. In one group (high-dose group, n=5), the infusion rate of 1% propofol was 2 ml h^{-1} (~60 mg kg^{-1} h^{-1}). In the second group (low-dose group, n=5), the infusion rate of 0.2% propofol, which was adjusted using normal saline to administer a similar fluid volume as that of the high-dose group, was 2 ml h^{-1} (~12 mg kg^{-1} h^{-1}). The intrarectal temperature was maintained at 37.5 (0.3)°C with a heating pad during the experimental period.

NMR measurements

A 2.0 T CSI Omega System equipped with an actively shielded gradient coil, Acustar S-150 (Bruker, Fermont, CA), was used in this study. A solenoid-type volume coil, 70 mm in diameter for transmission, and a surface coil 22 mm in diameter for signal detection was combined perpendicularly.

Brain lactate and *N*-acetylaspartate (NAA) were detected using the ^{1}H echo planar spectroscopic imaging (EPSI). The pulse sequence used in this study was described previously.[6 15] Measurements were acquired with 2 s repetition time (TR), 136 ms echo time (TE), 160 ms inversion time, 40 mm^2 field of view (FOV), 6 mm slice thickness at the centre of the brain, 4 k block size (16 points×256 echoes), 32 kHz spectral width, and 20 acquisitions for 10 phase encoding steps. The total acquisition time of one dataset of EPSI was 7.5 min. The EPSI data were Fourier transformed after zero filling, two 512×32×32 spectral datasets were obtained and added, and the metabolite images were constructed with 32×32 matrices.

Oedema was detected using diffusion-weighted echo planar imaging (EPI). The EPI was acquired with 3 s TR, 80 ms TE, 40 mm^2 FOV, 128×128 resolution, 3 mm slice thickness at the centre of the brain in the axial plane, and acquisitions. Diffusion gradients of 1290 and 2180 s mm^{-2} b values were applied separately on each X, Y and Z axis. The total acquisition time of one dataset of diffusion-weighted EPI was 2.5 min. Processing of NMR data was performed as described previously.[6] Briefly, two diffusion trace images with 1290 and 2180 s mm^{-2} b values were constructed by averaging the three diffusion-weighted EPIs with the X, Y, and Z axis gradients.[16] From these two trace images and the EPI without diffusion gradient, the average diffusion coefficient (Dav) was calculated pixel-by-pixel, and the Dav map was constructed. As the Dav values decrease along with the oedema formation, it is considered that the lower the values, the more severe the oedema.[16 17]

Experimental procedure

The experimental procedure is shown in Figure 1. Briefly, 30 min after starting propofol infusion, baseline measurements of the arterial blood gas parameters and blood glucose concentrations were performed. One dataset of diffusion-weighted EPI and EPSI at the baseline was then acquired. Pre-ischaemic hyperglycaemia was induced by i.p. injection of 20% glucose solution (2 g kg^{-1} glucose). Thirty minutes after glucose injection, arterial blood gas parameters and blood glucose concentrations were measured and MCAO on the left side was accomplished by advancing the tip of the occluding device into the middle cerebral artery. Immediately, diffusion-weighted EPI and EPSI were acquired alternately and repeatedly with a 10 min cycle. Sixty minutes after MCAO, reperfusion was induced by withdrawing the occluder to the extracranial internal carotid artery. Data acquisition was continued and repeated for the subsequent 120 min reperfusion period.

Statistical analysis

Values are expressed as the mean (SD). Repeated measures analysis of variance followed by the Bonferroni test were used for the comparison within group and between groups. A P value <0.05 was considered significant.

Results

Physiological data

Physiological parameters for each group are summarized in Table 1. In both groups, blood glucose concentration at the onset of ischaemia was elevated following injection of

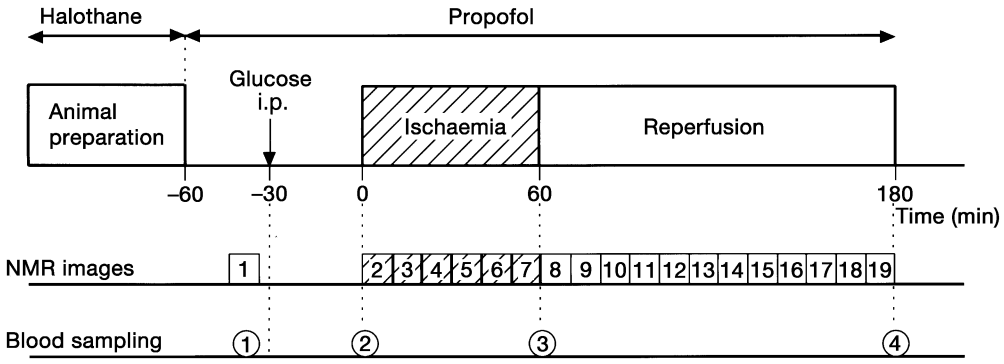

Fig 1 Experimental procedure for the NMR measurements. Arterial pressure measurement and blood sampling were performed before glucose injection, at the onset of ischaemia, at the onset of reperfusion and 120 min after reperfusion. The NMR images were obtained once before glucose injection, six times during ischaemia and 12 times after reperfusion.

Table 1 Physiological values for the two groups. Values are mean (SD) (n=5 in each group); *P<0.05 compared with baseline within each group

	High dose	Low dose
Baseline		
Mean arterial pressure (mm Hg)	124 (15)	125 (24)
Blood glucose (mg dl^{-1})	108 (18)	108 (20)
Arterial pH	7.37 (0.02)	7.36 (0.07)
Pa_{CO_2} (mm Hg)	44.4 (3.5)	44.1 (3.0)
Pa_{O_2} (mm Hg)	233 (19)	214 (20)
Onset of ischaemia		
Mean arterial pressure (mm Hg)	140 (13)	130 (15)
Blood glucose (mg dl^{-1})	249 (20)*	255 (24)*
Arterial pH	7.35 (0.03)	7.34 (0.03)
Pa_{CO} (mm Hg)	42.0 (4.7)	42.0 (4.4)
Pa_{O_2} (mm Hg)	221 (25)	235 (17)
Onset of reperfusion		
Mean arterial pressure (mm Hg)	112 (13)	132 (11)
Blood glucose (mg dl^{-1})	98 (15)	112 (17)
Arterial pH	7.34 (0.03)	7.34 (0.02)
Pa_{CO_2} (mm Hg)	44.7 (2.7)	41.5 (4.7)
Pa_{O_2} (mm Hg)	216 (25)	231 (24)
120 min after reperfusion		
Mean arterial pressure (mm Hg)	80 (18)*	118 (29)
Blood glucose (mg dl^{-1})	70 (5)*	73 (11)*
Arterial pH	7.40 (0.03)	7.39 (0.05)
Pa_{CO_2} (mm Hg)	44.0 (4.3)	43.1 (2.3)
Pa_{O_2} (mm Hg)	201 (25)	218 (38)

glucose but this concentration decreased below baseline 120 min after reperfusion in both the high-dose propofol and the low-dose propofol groups. Mean arterial pressure 120 min after reperfusion in the high-dose propofol group was significantly lower than baseline. There were no significant differences in arterial blood gas parameters and blood glucose concentrations between the groups at all sampling points. Mean arterial pressure 120 min after reperfusion in the high-dose propofol group was lower than that in the low-dose propofol group, but the difference was not significant.

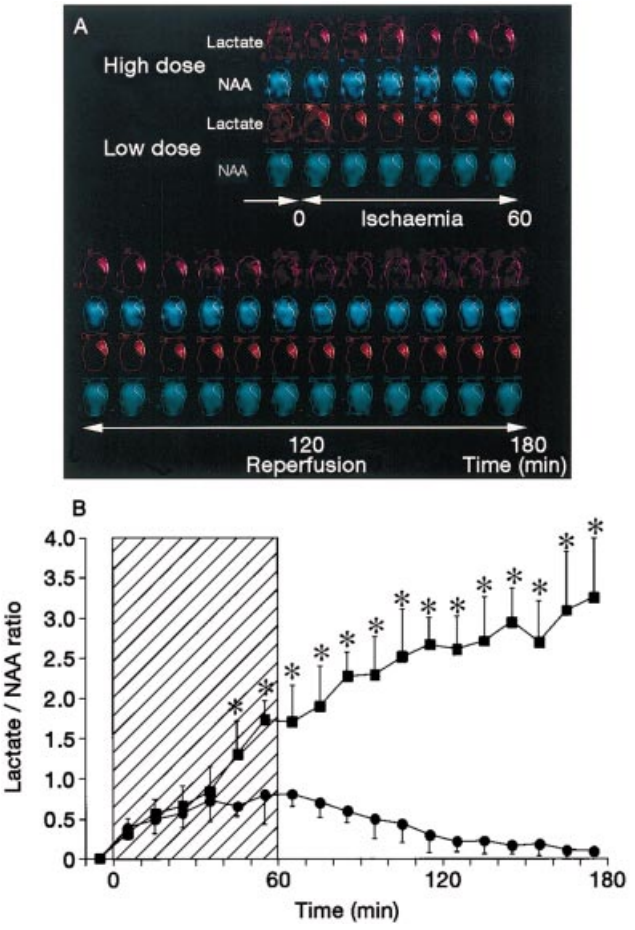

Fig 2 Effects of propofol on lactate accumulation. (A) Representative lactate and NAA images. (B) Changes in lactate/NAA ratio in the ischaemic region of the high-dose (●) and low-dose (■) propofol groups. The hatched area represents ischaemic period. Values are mean (SD) (n=5 in each group). Asterisks denote significant difference between the high-dose and low-dose propofol groups at the same time points (*P<0.05).

Lactate accumulation

Images of lactate accumulation are shown in Figure 2A. After the onset of ischaemia, lactate accumulation was detected in the ischaemic region both in the high-dose propofol group and the low-dose propofol group. After reperfusion, the lactate accumulation disappeared gradually

in the high-dose propofol group, while it did not decline in the low-dose group. To evaluate the lactate accumulation quantitatively, the lactate/NAA ratio was calculated from the spectra extracted from the ischaemic region. As NAA is distributed through the brain in neurons but not in glia, it can be used as a marker of neuronal density.[18] As shown in Figure 2B, the lactate/NAA ratio increased from the onset of ischaemia until 40 min after ischaemia similarly in both groups. However, in the high-dose propofol group, no further increase was observed and the ratio decreased gradually to the baseline concentration after reperfusion. In the low-dose propofol group, the ratio increased continuously during ischaemia and reperfusion.

Oedema formation

Images of the oedema formation are shown in Figure 3A. Oedema gradually appeared in the ischaemic hemisphere after the onset of ischaemia both in the high-dose and low-dose propofol groups. However, during reperfusion, oedema disappeared in the high-dose propofol group, whereas it did not decline in the low-dose propofol group. To evaluate oedema size quantitatively, the number of pixels with an average diffusion coefficient (Dav) below 0.6×10^{-3} mm^2 s^{-1}, which can be considered as the formation of oedema,[6] was counted from the left hemisphere. During ischaemia, the pixel number increased to a similar extent in both groups. After reperfusion, the pixel number in the high-dose propofol group decreased and returned to the baseline level, whereas that in the low-dose propofol group continued to increase (Fig. 3B).

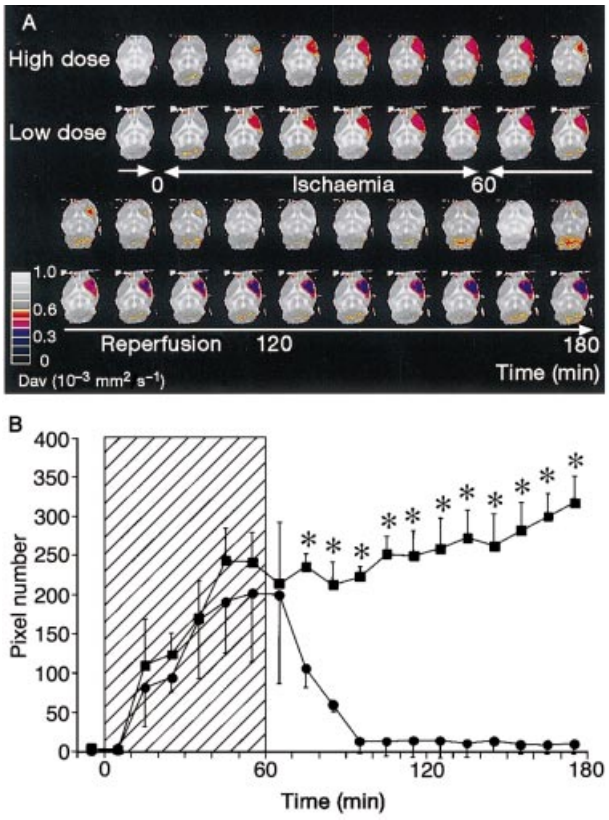

Fig 3 Effects of propofol on oedema formation. (A) Representative average diffusion coefficient (Dav) maps. Dav values below 0.6×10^{-3} mm^2 s^{-1} are shown with colours, which means oedema formation. (B) Changes in pixel number with Dav values below 0.6×10^{-3} mm^2 s^{-1} of the high-dose (●) and low-dose (■) propofol groups. The hatched area represents ischaemic period. Values are mean (SD) (n=5 in each group). Asterisks denote significant difference between the high-dose and low-dose propofol groups at the same time points (*P<0.05).

Discussion

In the present investigation, the NMR study revealed that high-dose administration of propofol attenuated the accumulation of lactate and the formation of oedema in the ischaemic region in hyperglycaemic rats.

We used SHRs for the MCAO model, because the MCAO-induced infarction in SHRs is larger in volume and more reproducible than that in other strains of rats.[19] I.p. injection of glucose produced higher blood glucose levels than controls at the onset of ischaemia, but this was lower than controls 120 min after reperfusion. In studies using diabetic rats, it was reported that the severity of the brain ischaemia depended not on the duration of hyperglycaemia, but on the blood glucose level at the onset of the ischaemia.[20,21] Therefore, the time course of the blood glucose changes in the present study can be considered sufficient to aggravate neuronal damage induced by brain ischaemia.

Ischaemia, as well as hypoxia, causes inhibition of aerobic metabolism, leading to production and accumulation of lactate, which induces tissue acidosis and cellular damage.[4,22] Hyperglycaemia-induced elevation of brain glucose concentration should accelerate lactate accumulation and aggravate ischaemia-induced neuronal damage.[5]

Propofol has been already shown to exert neuroprotective effects in cerebral ischaemia in normoglycaemic rats;[8–10] however, it has not been shown whether propofol can prevent ischaemia exacerbated by hyperglycaemia. Propofol has been shown to have profound depressive effects on cerebral glucose metabolism in rats and humans.[11,12] In rats, propofol inhibited the cerebral glucose utilization in a dose-dependent manner.[12] Therefore, in the present study, we suggest that high-dose propofol suppresses glucose metabolism more profoundly than low-dose propofol, which lowered lactate accumulation in response to ischaemia.

Reactive oxygen intermediates have been implicated in the pathophysiology of ischaemia-induced brain damage.[23,24] Hyperglycaemia accentuates acidosis in the ischaemic region, which enhances formation of hydroxyl radicals, resulting in the exacerbation of ischaemia-induced brain damage.[25,26] The chemical structure of propofol

resembles that of α-tocopherol, an antioxidant.[27] Indeed, the antioxidant properties of propofol have been reported.[28 29] Therefore, the neuroprotective effect of propofol in hyperglycaemic rats may be partially attributable to its antioxidative properties.

In the present study, neuroprotective effects were observed with a relatively high-dose of propofol (60 mg kg^{-1} h^{-1}). This propofol dose roughly corresponds to that which produced burst suppression on electroencephalograms in rats.[8 9] In humans, 15–30 mg kg^{-1} h^{-1} of propofol was reported to give rise to burst suppression in electroencephalograms.[30] Therefore, the findings of the present study suggest that 15–30 mg kg^{-1} h^{-1} of propofol may be neuroprotective in brain ischaemia in diabetic patients. However, further studies will be required to clarify the neuroprotective effects in humans.

In conclusion, high-dose propofol attenuated lactate accumulation and oedema formation in cerebral ischaemia in hyperglycaemic rats. As lactate accumulation is considered to exacerbate brain damage, the use of propofol during neurosurgical operations may be beneficial to prevent neuronal damage especially in diabetic patients.

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