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Research report

Simvastatin reduced ischemic brain injury and perfusion deficits in an embolic model of stroke

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Abstract

Simvastatin is cholesterol lowering agent and also a modulator of cytokine in the nervous system. The functional significance and neuroprotectiove mechanism of simvastatins in ischemic brain injury is controversial. The purpose of study is to evaluate the effect of simvastatin on ischemic brain injury and to investigate the perfusion capability of brain microvessels in the ischemic injury. This study included two series of experiments. In the first series, we studied if simvastatin is neuroprotective in an embolic model of stroke. The treatments began 2 weeks before middle cerebral artery (MCA) occlusion. Infarct volume was measured at 48 h post stroke. Neurological deficits were assessed at 2 h, 24 h and 48 h post stroke. Results showed that infarct volume in rats which received saline and simvastatin was 32.5 ± 9.3% (mean ± SD) and 18.7 ± 6.5%, respectively. The infarct volume in the simvastatin group was significantly smaller than in the controls ($P < 0.002$). Treatment with simvastatin also improved neurological deficits and reduced brain edema significantly ($P < 0.05$). In the second series, we studied if simvastatin can improve microvascular reperfusions after ischemia. Perfusion deficits were detected at 8 h post stroke using Evens blue dye. Neurological deficits were assessed at 2 h and 8 h post stroke. Results showed that perfusion deficit in saline and simvastatin-treated groups were 58.7 ± 8.7% and 23.4 ± 7.5%, respectively. The perfusion deficit in simvastatin-treated group was decreased 61% ($P < 0.01$). These studies thus suggest that simvastatin is a protective agent in ischemic brain injury and this protective effect may be partially due to its action in the improvement of microvascular reperfusion.

Theme: Disorders of the nervous system
Topic: Ischemia

Keywords: Simvastatin; Embolization; Perfusion deficits; Ischemic brain injury

1. Introduction

In patients with ischemic brain injury, the occluded artery often reopens over time. This reopening may result through a natural dissolution of the occluding material and fragments of the material may moved down stream to obstruct distal arteries. Thrombolysis results in an improvement in clinical outcome were used in patients and experimental models of focal cerebral ischemia. Later, it was reported that tissue plasminogen activator was effective in opening coronary arteries, its usefulness in restoring cerebral blood flow was tested. Nowadays, research showed that simvastatin may be effective like tissue plasminogen activator (t-PA) for treatment of ischemic brain injury. Simvastatin, 3 hydroxy-3 methylglutharyl coenzyme A reductase inhibitor, is the most widely used as cholesterol-lowering drug [2,10]. Simvastatin is also a cytokines modulator and Na^+/K^+ pump current modulator [11,14], upregulator of endothelial NO synthase (eNOS), which may correspond to a mechanism against cerebrovascular injury [2,23]. However, data accumulated indicate that the functional significance of neuroprotective effect of simvastatin in stroke is controversial [1,7]. In 1990s, it was reported that simvastatin acts as a mediator of central nervous system (CNS) cell death [13]. These observations were confirmed later by Crick et al. [5] and Parsanna et al. [15]. In the present

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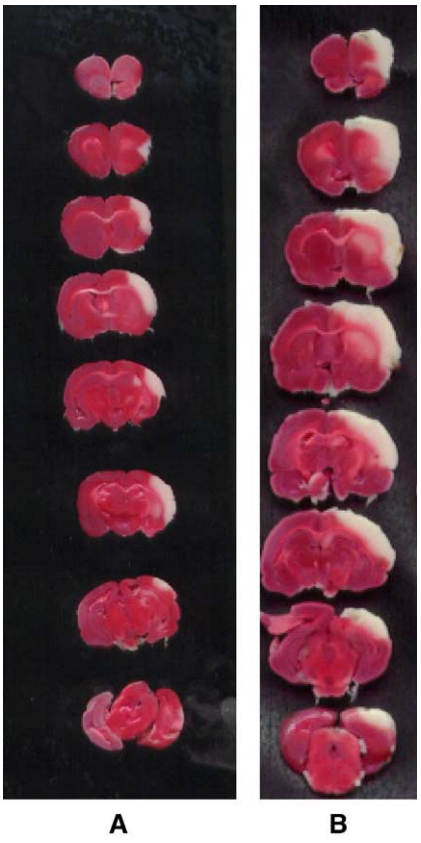

Fig. 1. Representative TTC-stained brain sections from different groups. (A) A rat from simvastatin group, and (B) a rat from control group.

study, we examined the neuroprotective effects of simvastatin in ischemic brain injury using a clinical relevant model of stroke in rats. Furthermore, we also examined the effects of simvastatin on perfusion deficits in ischemic brain injury.

2. Materials and methods

Male Sprague–Dawley rats with weighting 250–300 g were used. Animal care and the general protocols for animal use were approved by the Animal Ethics Committee of the University of Alberta. There were two series of experiments in this study. In the first series, the effect of simvastatin on infarct volume and functional recovery were examined. Animals were randomly assigned into control group (n = 10) or simvastatin (n = 10) treatment group. In the control group, animals received saline and in the second group, the rats were treated with simvastatin, 100 mg/kg, once per day. The treatments began 2 weeks before middle cerebral artery (MCA) occlusion. In the second series of experiments, the effects of simvastatin on perfusion deficits were studied in the ischemic injured brain. The treatment was the same as in the first experiment, that is, rats received either saline or simvastatin. All of drugs were administered by intraperitoneal injection.

Table 1
Neurological deficits in experiment 1[a]

Group	2 h	24 h	48 h
Saline	3 (2–3.25)	3 (2–4)	3 (2–4)
Simvastatin	3 (3–4)	2.5 (2–3)[b]	2 (2–3)[b]

[a] The neurological deficits scores are expressed as median and interquartile ranges, the 25–75th percentile are shown in the parenthesis.
[b] Denotes significantly different from control group.

2.1. Cerebral focal ischemia model

Focal cerebral ischemia was induced by embolizing a preformed clot into the MCA, as reported previously [17,20,21]. In brief, the rats were initially anesthetized with 3.0% halothane and then maintained with 1.5% halothane in a mix of O_2 and NO_2 during surgery. Body temperature was maintained at 37 °C with a heating pad for the duration of surgery and immediate post-operation period until the animal recovered fully from anesthesia. A longitudinal incision of 1.5 cm in length was made in the midline of the ventral cervical skin. The right common

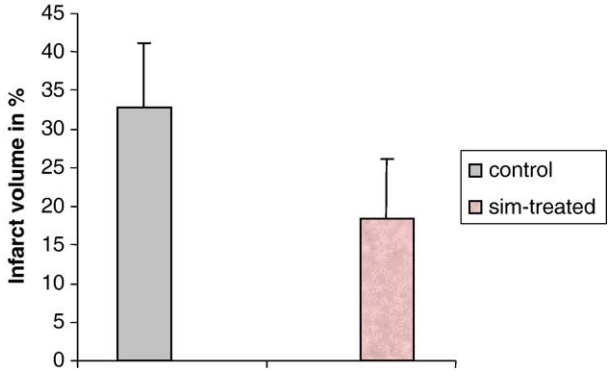

Fig. 2. Infarct volume changes in different groups. Infarct volumes were measured at 48 h after MCA occlusion. Compared with the control group, treatment with simvastatin reduced infarct volume significantly (P < 0.002).

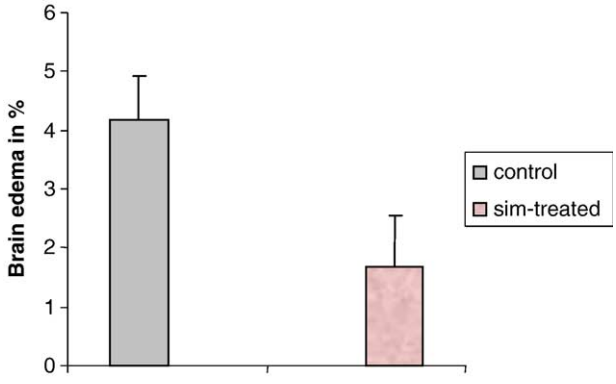

Fig. 3. Effects of saline or simvastatin on brain swelling. Brain swellings were measured at 48 h after MCA occlusion. Compared with the control group, treatment with simvastatin improved brain swelling significantly (P < 0.001).

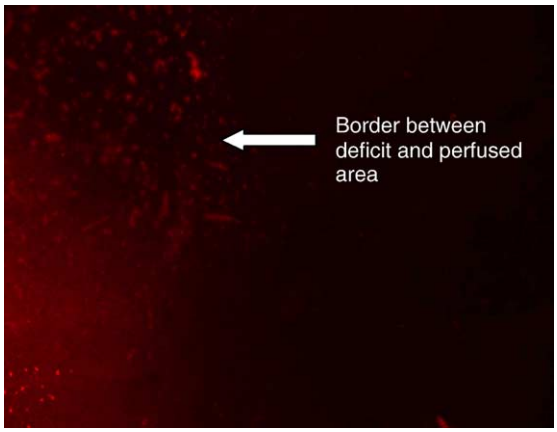

Fig. 4. Figure shows the borderline between perfused area (black zone) and perfusion deficits area (red zone). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

carotid artery (CCA), right internal carotid artery (ICA) and right external carotid artery (ECA) were exposed. The distal portion of the ECA was ligated and cut. A modified PE-10 catheter, filled with bovine thrombin (Thermostat, TM Warner-Lambert Co., Scarborough, Canada), was introduced into the lumen of the right ECA via a small puncture. Ten microliters of blood were withdrawn into the catheter and retained for 15 min to allow formation of a clot. Once the clot formed, the catheter was advanced 17 mm in the ICA until its tip was 1–2 mm away from the origin of the MCA. The preformed clot in the catheter was then injected and the catheter was removed. The wound was closed and the animal returned to its cage. The dynamic changes of the microvessel occlusion in this model have been characterized [22].

2.2. Quantification of brain infarct volume

The quantification of infarct volume has been detailed previously [21]. Briefly, 48 h after MCA occlusion, anesthetized rats were decapitated and the brains were removed. For morphometric study, 2-mm-thick coronal sections were cut using a rat brain matrix. A total of 8 coronal sections were collected and the sections were stained using a 2% 2, 3, 5-triphenyltetrazolium chloride (TTC) solution. The stained brain sections were scanned with color flatbed scanner. The images were analyzed by a person who was unaware of the treatments, using a commercial image processing software-program, Photo-Shop. The total volume of each hemisphere and infarction was determined by integration of the areas from the eight sections. The infarct volume was calculated with following formula: infarct volume = [the volume of the left hemisphere − (the volume of the right hemisphere − measured infarct volume)] / the volume of the left hemisphere [18]. The infarction volume was expressed in percentage.

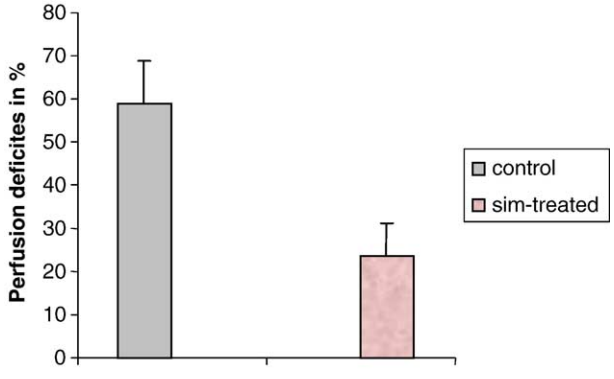

Fig. 5. Effects of saline or simvastatin on the perfusion deficits of brain at 8 h after embolization. Compared with the group received saline treatment, the deficits were significantly lower in simvastatin-treated group ($P < 0.01$).

2.3. Quantification of perfusion deficits

Perfusion deficits of microvessels were determined using Evans blue dye, as described previously with modification [22,24]. 2% Evans blue solution, 0.2 ml/100 g of body weight, was injected to tail vein before MCA occlusion. Eight hours after embolization, saline was administrated to the left ventricle in the rate of 120 ml/min to wash out Evans blue dye in the blood circulation system, and Evans blue dye in the regions where the feeding vessels occluded still remained. The animals were decapitated and their brain removed and stored at −70 °C until sectioning. The brains were sectioned at 10 μm in thickness with a cryostat beginning 3.7 mm rostral to the bregma. For each brain, 9 consecutive sections, with 1 mm interval, were collected. As described in the study from Zhang et al. [24] and our own study [22], individual blood vessel in the perfusion deficits was detected by red fluorescence.

2.4. Behavioral test

Neurological deficits and seizure activities were recorded at 2, 24 and 48 h after embolization in the first series of experiments and at 2 and 8 h in the second series. Neurological deficits were determined using a modified Bederson's scoring system [3]. 0: no observable deficit; 1: forelimb flexion; 2: forelimb flexion plus decreased resistance to lateral push; 3: unidirectional circling; 4: unidirectional circling plus decreased level of consciousness. Seizure activities were evaluated by Racine's scoring method [16].

Table 2
Neurological deficits in experiment 2[a]

Group	2 h	8 h
Saline	3 (2.75–4)	3 (2–4)
Simvastatin	3 (2–3.25)	2 (2–3)[b]

[a] The neurological deficits scores are expressed as median and interquartile ranges, the 25–75th percentile are shown in the parenthesis.
[b] Denotes significantly different from control group.

2.5. Statistical analysis

The data were analyzed by stat view. The comparison of brain infarct volume, and perfusion deficits were analyzed by independent sample t test. Before using the above test, the homogeneity of variances and distributions of data were evaluated. The comparisons of neurological scores (median) were analyzed with Mann–Whitney U test and expressed with interquartile range. The comparisons of seizure were analyzed with Chi square test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Experiment 1

3.1.1. Infarct volume

Infarct volume at 48 h after MCA occlusion is shown in Fig. 1. In the control group, infarct volume was 32.5 ± 9.3% (mean ± SD). The infarct volume was 18.7 ± 6.5% in the group received treatment with simvastatin. Compare to the control group, simvastatin reduced infarct volume by 51% ($P < 0.002$), (Fig. 2). In addition, treatment with simvastatin also significantly reduced edema in the ischemic injured brain (Fig. 3).

3.1.2. Behavioral tests

At 2, 24 and 48 h in control group and at 2 h in simvastatin-treated group after MCA occlusion, all animals showed significant motor deficits with median score of 3 (Table 1). At 24 and 48 h after MCA occlusion median score of neurological deficits was 2.3 and 2, respectively in simvastatin-treated group. Treatment with simvastatin significantly improved the neurological deficits ($P < 0.05$). Seizure activity was also observed in 3 rats in control group and 2 rats in the simvastatin group.

3.2. Experiment 2

3.2.1. Perfusion deficits

Perfusion deficits changes at 8 h after MCA occlusion are shown in Fig. 4. In the control group, perfusion deficits were 58.7 ± 8.7% (mean ± SD). Compared to the control group (Fig. 5), treatment with simvastatin (23.4 ± 7.5%) reduced perfusion deficits significantly ($P < 0.01$). Simvastatin treatment reduced perfusion deficit by 61%, compared with that in the controls.

3.2.2. Behavioral tests

At 2 and 8 h in control group and at 2 h in simvastatin-treated group after MCA occlusion, all animals showed significant motor deficits with median score of 3 (Table 2). At 8 h after MCA occlusion median score of neurological deficits was 2 in simvastatin-treated group. Treatment with simvastatin significantly improved the neurological deficits ($P < 0.05$). Seizure activity was also observed in 2 rats in control group and 2 rats in simvastatin treated group.

4. Discussion

In the present studies, we examined if simvastatin treatment can improve recovery of ischemic brain injury, and if re-establishment of recirculation in the injured brain play a role in this recovery. Results from the first series of experiments showed that treatment with simvastatin significantly reduced the infraction in the brain. Treatment with simvastatin also significantly improved functional recovery, measured with the changes of neurological deficits. Results from the second series of experiments showed that treatment with simvastatin significantly reduced perfusion deficits, and also improved functional recovery.

The outcomes therefore are in agreement with previous findings that simvastatin plays a significant role as a neuroprotective agent after MCA occlusion [7,8,19]. Since we did not measure intermediate metabolites effects of simvastatin and cholesterol level, we were unable to definitely determine if decreases of perfusion deficits, infarct volume, brain edema and neurological deficits improvement are due to increasing NOS and antioxidant activity or reducing cholesterol level. It may also be possible that simvastatin acts on other system such as cardiovascular system which in turn causes protective effect on cerebrovascular system. The protective actions of treatment with simvastatin, however, might also be via several other mechanisms. 1. Anti-inflammatory effects in consequence of reducing acute phase proteins, including C-reactive protein, inflammatory cytokines and cell adhesion molecules. Particularly simvastatin's ability to downregulate endothelial cell activation induced by different stimuli, strongly suggests their possible use in pathogenic conditions [12]. 2. Antioxidant effects because of scavenging of superoxide and inhibition of isoprenoids (superoxide generators) [6,11]. 3. tPA-like effects due to a shift in the fibrinolytic balance towards fibrinolysis and reduced platelet aggregation [4,9,12].

References

[1] W. Balduini, V. De Angelis, E. Mazzoni, M. Cimino, Simvastatin protects against long-lasting long lasting behavioral and morphological consequences of neonatal hypoxic/ischemic brain injury, Stroke 32 (2001) 2185–2191.

[2] W. Balduini, E. Mazzoni, S. Caloni, M.G.D. Simoni, Prophylactic but not delayed administration of simvastatin protects against long-lasting cognitive and morphological consequences of neonatal hypoxic/ischemic brain injury, Stroke 34 (2003) 2007–2012.

[3] J.B. Bederson, L.H. Pitts, M. Tsuji, M.C. Nishimora, R.L. Davis, Rat middle cerebral artery occlusion: evaluation of the model and development of a neurological examination, Stroke 17 (1986) 472–476.

[4] T. Bourcier, P. Libby, HMG-CoA reductase inhibitors reduce plasminogen activator inhibitor-1 expression by human vascular

smooth muscle and endothelial cells, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 556–562.
[5] D.C. Crick, D.A. Andres, R. Danesi, M. Macchia, C.J. Waechter, Granylgraniol overcomes the blocks of cell proliferation by lovastatin in C6 glioma cells, J. Neurochem. 70 (1998) 2397–2405.
[6] A.P. Day, S. Belavia, O.T.G. Jones, D. Stansbie, Effect of simvastatin therapy on cell membrane cholesterol content and membrane function as assessed by polymorphonuclear cell NADPH oxidase activity, Ann. Clin. Biochem. 34 (1997) 269–275.
[7] D. Duval, Effects of statins on ischemic stroke: neuroprotection and/or triggering of apoptotic damage, Stroke 31 (4) (2000) 989–990.
[8] M. Endres, U. Laufs, Z. Huang, T. Nakumura, P. Huang, Stroke protection by 3-hydroxy3methylglutharyl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase, Proc. Natl. Acad. Sci. U. S. A. 959 (15) (1998) 8880–8885.
[9] D. Ferro, S. Basili, C. Alesandri, D. Cara, F. Violi, Inhibition of tissue factor mediated thrombin generation by simvastatin, Atherosclerosis 149 (2000) 111–116.
[10] M.R. Law, N.J. Wald, A.R. Rudnica, Quantifying effect of statins on low density lipoprotein cholesterol, ischemic heart disease, and stroke: systematic review and metaanalysis, BMJ 326 (2003) 1407–1408.
[11] A.M. Lefer, R. Scalia, D.J. Lefer, Vascular effects of HMGA-reductase inhibitors (statins) unrelated to cholesterol lowering: new concept of cardiovascular disease, Cardiovasc. Res. 49 (2001) 281–287.
[12] P. Meroni, C. Luzzana, D. Ventura, Anti-I inflammatory and immunomodulating properties of statins: an additional tool for therapeutic approach of systemic autoimmune disease? Clin. Rev. Allergy Immunol. 23 (3) (2002) 263–278.
[13] M. Michikawa, Y. Yanagisawa, Apolipoprotein E4 induces neuronal death under conditions of suppressed de no cholesterol biosynthesis, J. Neurosci. Res. 54 (1998) 58–67.
[14] K. Pahan, F.G. Sheikh, A.M. Namboodiri, I. Singh, lovastatin and phenyl acetate inhibit the induction of nitric oxide synthase and cytokines in rat primary asterocytes, microglia, and macrophages, J. Clin. Invest. 100 (1997) 2671–2679.
[15] P. Prasnna, A. Thibault, L. Liu, D. Samid, Lipid metabolism as a target for brain cancer therapy: synergistic activity of lovastatin and sodium phenyl acetate against human glioma cells, J. Neurochem. 55 (1996) 710–716.
[16] R. Racine, Modification of seizure activity by electrical stimulation, II: motor seizure, Electroencephalogr. Clin. Neurophysiol. 32 (1972) 281–294.
[17] A.P. Shabanzadeh, A. Shuaib, T. Yang, S. Abdul, C.X. Wang, Effect of zinc in ischemic brain injury in an embolic model of stroke in rats, Neurosci. Lett. 356 (2004) 69–71.
[18] A. Shuaib, C.X. Wang, T. Yang, R. Noor, Effects of nonpeptideV1 vasopressin receptor antagonist SR-49059 on infarction volume and recovery of function in a focal embolic stroke model, Stroke 33 (2002) 3033–3037.
[19] C.J. Vaughan, N. Delanty, Neuroprotective properties of statins in cerebral ischemia and stroke, Stroke 30 (1999) 1969–1973.
[20] C.X. Wang, Y. Yaung, A. Shuaib, An improved version of embolic model of brain ischemic injury in the rat, J. Neurosci. Methods 109 (2001) 147–151.
[21] C.X. Wang, Y. Yaung, T. Yaung, A. Shuaib, A focal embolic of cerebral ischemia in rats: introduction and evaluation, Brain Res. Protoc. 7 (2001) 115–120.
[22] C.X. Wang, K.G. Tod, Y. Yaung, T. Gordon, A. Shuaib, Patency of cerebral microvessels after focal embolic stroke in the rat, Cereb. Blood Flow Mech. 21 (2001) 413–421.
[23] M. Yamada, Z. Huang, T. Dalkara, M. Endres, U. Laufs, Endothelial nitric oxide synthase-dependent cerebral blood flow augmentation by L-arginine after chronic statin treatment, Cereb. Blood Flow Metab. 20 (2000) 709–717.
[24] R.L. Zhang, M. Chopp, Z.G. Zhang, Q. Jiang, J.R. Ewing, A rat model of focal embolic cerebral ischemia, Brain Res. 766 (1997) 83–92.