Acta Neurochir (Wien) (2000) 142: 193–197

Acta Neurochirurgica

Printed in Austria

Delayed Administration of the K^+ Channel Activator Cromakalim Attenuates Cerebral Vasospasm after Experimental Subarachnoid Hemorrhage

A.-L. Kwan[1,2], **C.-L. Lin**[1], **C.-S. Wu**[1], **E.-F. Chen**[2], **S.-L. Howng**[1], **N. F. Kassell**[2], and **K. S. Lee**[2,3]

[1] Department of Neurosurgery, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[2] Department of Neurosurgery, University of Virginia, Charlottesville, VA
[3] Department of Neuroscience, University of Virginia, Charlottesville, VA

Summary

Background. Delayed cerebral vasospasm remains an unpredictable and inadequately treated complication of aneurysmal subarachnoid hemorrhage (SAH). Recent evidence indicates that the potassium channel activator cromakalim is capable of limiting cerebral vasospasm in rabbits when administered immediately after experimental SAH (i.e. before spastic constriction has been initiated). However, the ultimate clinical value of cromakalim for treating vasospasm will depend in part on its effectiveness when administered after SAH-induced constriction has already been initiated. The present study examined the effects of cromakalim on vasospasm when treatment was initiated after SAH-induced constriction was underway.

Methods. New Zealand white rabbits were subjected to experimental SAH by injecting autologous blood into the cisterna magna. Cromakalim (0.03, 0.1 or 0.3 mg/kg) or vehicle was injected intravenously at 8 hour intervals beginning 24 hours post-SAH. Animals were killed by perfusion fixation 48 hours after SAH. Basilar arteries were removed and sectioned, and cross-sectional area was measured.

Findings. The average cross sectional areas of basilar arteries were reduced by 64% and 68% in the SAH-only and SAH + vehicle groups, respectively. Treatment with cromakalim dose-dependently attenuated SAH-induced constriction. The groups treated with 0.03, 0.1, and 0.3 mg/kg cromakalim exhibited average decreases in cross-sectional area of 57%, 42%, and 19%, respectively.

Interpretation. These findings indicate that cromakalim dose-dependently attenuates cerebral vasospasm when administered 24 hours after experimental SAH in the rabbit. The results suggest K_{ATP} channel activators, such as cromakalim, could be of benefit for reversing cerebral vasospasm after aneurysmal SAH.

Keywords: Cromakalim; potassium channel; subarachnoid hemorrhage; vasospasm.

Introduction

Cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH) is the leading cause of death and disability in patients who survive the initial effects of aneurysmal rupture [4–6]. Despite substantial research, the pathogenesis of cerebral vasospasm remains a matter of discussion, and adequate pharmacotherapy has been elusive. Consequently, it is still of considerable importance to identify and evaluate potential mechanisms and treatments for cerebral vasospasm.

In a review article that appeared in 1994, Zhang and Cook [24] suggested that potassium channel openers might be of value in the management of cerebral vasospasm. The rationale for this argument was that the opening of potassium channels would lead to the hyperpolarization of vascular smooth muscle cells, resulting in a relaxation of the contractile state of the cells. Studies by Zuccarello and associates [26] have provided important support for this concept. Using electrophysiological recording techniques, rabbit smooth muscle cells obtained from spastic basilar arteries were shown to be depolarized, and this depolarization could be reversed by the ATP-sensitive potassium channel (K_{ATP}) opener cromakalim. These findings suggest that selective potassium channel modulators can be effective in altering the polarization of smooth muscle cells that have been compromised by SAH. The same authors further examined this issue by topically applying cromakalim to intact basilar arteries that had been subjected to experimental SAH [26]. In these experiments, cromakalim reversed an established arterial narrowing observed 3 days after SAH. Although the time course of SAH-induced vascular narrowing in the rabbit is not as prolonged as that observed in human patients, this observation raised the possibility that post-hemorrhagic treatment with a potassium channel opener could be beneficial

for reversing cerebral vasospasm. Recently, we have demonstrated that systemically administered cromakalim is effective in blocking the development of basilar artery vasospasm in a rabbit model of experimental SAH [9]. A dose-dependent protective effect of intravenously administered cromakalim was obtained when treatment was initiated one hour after experimental SAH. Treatment with cromakalim prior to the establishment of profound vascular narrowing is of potential clinical value in the prophylactic treatment of SAH patients deemed to be at risk for developing cerebral vasospasm [9]. However, it would be of considerable additional benefit if systemic cromakalim treatment were to be effective when initiated after substantial arterial narrowing had already been established. The goal of the present study was to examine this issue by using a protocol in which intravenous cromakalim treatment was initiated after SAH-induced vascular narrowing has been established.

Materials and Methods

Experimental Subarachnoid Hemorrhage (SAH)

All experimental protocols were approved by the University of Virginia Animal Research Committee. New Zealand White rabbits (3.2–4.0 kg) were subjected to an experimental subarachnoid hemorrhage (SAH) by injecting autologous blood into the cisterna magna. Animals were anesthetized with an intramuscular injection of a mixture of 9 mg/kg xylocaine and 55 mg/kg Ketamine and then intubated endotracheally. Three milliliters of arterial blood were withdrawn from the ear artery, and injected into the cisterna magna with a 23-gauge butterfly needle after aspirating 1 ml of cerebrospinal fluid. The animals were positioned in ventral recumbency for at least 15 minutes to facilitate the formation of a blood clot in the subarachnoid cistern. All animals were monitored closely for respiratory distress, and ventilated as necessary. The animals were then allowed to recover from anesthesia, extubated when awake, and returned to the vivarium.

Treatment Groups

Sixty-four rabbits were divided into the following eight groups: 1) control (no SAH; n = 8); 2) control + vehicle (n = 8); 3) control + high dose cromakalim (0.3 mg/kg; n = 8); 4) SAH-only (n = 8); 5) SAH + vehicle (n = 8); 6) SAH + low dose cromakalim (0.03 mg/kg; n = 8); 7) SAH + medium dose cromakalim (0.1 mg/kg; n = 8) and 8) SAH + high dose cromakalim (0.3 mg/kg; n = 8). All injections of drug or vehicle were administered intravenously, and were initiated 24 hours after the induction of SAH. Substantial vascular constriction is already observed in the rabbit SAH model at the 24-hour time point [8]. Subsequent injections were given at 32 and 40 hours post-SAH.

Perfusion-Fixation

Forty-eight hours post-SAH, animals were reanesthetized, intubated, ventilated, and paralyzed with pancronium bromide (0.3 mg/kg). The central ear artery was cannulated in order to monitor blood pressure and to determine the blood gas levels. Perfusion-fixation was performed in the following manner. The thorax was opened, a cannula was placed in the left ventricle, the descending thoracic aorta was clamped, and the right atrium was opened. Perfusion was begun with 300 ml of Hank's Balanced Salt Solution (HBSS Sigma Chemical # H-1387), pH 7.4 at 37 °C, followed by 200 ml of a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in HBSS (pH 7.4 at 37 °C). Perfusion was performed at a pressure of 120 cm H_2O. Following perfusion-fixation, the brain was removed and immersed in the same fixative overnight at 4 °C.

Visual inspection during the removal of the brain showed that all animals had subarachnoid clots covering the basilar artery. Two animals were eliminated from the study at this point due to inadequate perfusion-fixation: one rabbit from the SAH + vehicle group, and one from the SAH + low dose cromakalim group.

Tissue Embedding

Arterial segments were removed from the middle third of each basilar artery and washed several times in 0.1 mol/L phosphate buffer (PBS; pH 7.4). The specimens were postfixed with osmium tetroxide, rinsed, dehydrated and embedded in Epon 812. Cross sections of basilar arteries were cut at a thickness of 0.5 μm with an ultramicrotome, mounted on glass slides and stained with toluidine blue for morphometric analysis.

Tissue Morphometry and Statistical Analysis

Morphometric measurements were performed by an investigator blinded to the treatment group to which the arteries belonged. Measurements were performed on at least five random arterial cross-sections using a computer-assisted image analysis system (Model Bx 50 F Olympus Optical Co, LTD). The area values for the five cross sections from a given artery were averaged to provide a single value for each animal. Group data are expressed as mean ± SEM. Group comparisons were performed using a one way analysis of variance (ANOVA) with the Tukey *post-hoc* test. Differences were considered significant at $P < 0.05$ level.

Results

General Observations

All animals exhibited a thick subarachnoid clot over the basal surface of the brain stem, which covered the basilar artery. There were no significant differences among the treatment groups for the physiological parameters recorded prior to perfusion-fixation (Table 1). Qualitative histological observations revealed substantial corrugation of the internal elastic lamina (IEL) of the basilar arteries in the SAH only and SAH + vehicle groups. Corrugation of the IEL was less prominent in the animals treated with cromakalim; animals in the high dose cromakalim group exhibited little or no corrugation of the IEL.

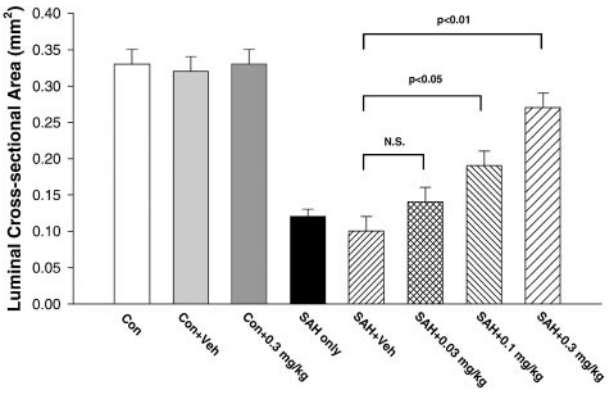

Fig. 1. Effect of cromakalim on SAH-induced cerebral vasospasm. Cromakalim elicited a dose-dependent reduction in SAH-induced vasospasm. *Con* Control; *Veh* vehicle; *SAH* subarachnoid hemorrhage; *NS* not significant

Cross-Sectional Area Measurements

The group averages for basilar artery area did not differ significantly among the control, control + vehicle, and control + high dose cromakalim groups (Fig. 1). Basilar artery cross sectional area was reduced in groups subjected to experimental SAH. The average areas were reduced from control levels by 64% and 68% in the SAH-only and SAH + vehicle groups, respectively (Fig. 1). The SAH-induced reduction in cross sectional area was attenuated in animals treated with cromakalim. The average areas were reduced from control levels by 57%, 42% and 19% in the SAH groups treated with 0.03, 0.1 and 0.3 mg/kg cromakalim, respectively (Fig. 1). The cross-sectional areas of the SAH + 0.1 mg/kg and SAH + 0.3 mg/kg groups were significantly greater than that of the SAH + vehicle group. The protective effect of the high dosage of cromakalim was such that the cross-sectional area of the SAH + 0.3 mg/kg group did not differ significantly from that of the control + vehicle group.

Discussion

Potassium channels play diverse roles in regulating vascular function [13, 22, 24]. Multiple subtypes of potassium channels are expressed in vascular smooth muscle, and the distribution of individual potassium channel subtypes can vary substantially among different vascular beds. The potassium channel opener used in the present study, cromakalim, preferentially activates the K_{ATP} channel [2, 3, 17, 20]. Activation of the K_{ATP} channel has a net hyperpolarizing effect on vascular smooth muscle cells, which can lead to a relaxation of the cell's contractile state. Cromakalim and other K_{ATP} channel openers have been shown to elicit vasodilation in large and small cerebral arteries [1, 7, 10, 11, 15, 16, 19, 23], indicating that K_{ATP} channels are present on a broad range of cerebral vessels. However, even though most cerebral vessels are responsive to cromakalim, the magnitude of the effect of K_{ATP} channel openers varies significantly among different cerebral vessels [14]. The possibility that modulators of the K_{ATP} channel could be beneficial in the treatment of cerebral vasospasm has been raised by several groups of investigators [9, 19, 24–26]. Zuccarello and associates [26] provided direct evidence for this concept by demonstrating that topically applied cromakalim is capable of dose-dependently reversing SAH-induced vascular constriction in rabbit basilar arteries. In addition, these authors demonstrated that cromakalim could reverse the depolarization of vascular smooth muscle cells that was induced by SAH. This effect of was blocked by an inhibitor of K_{ATP} channels (glyburide), a finding that further implicates K_{ATP} channels in post-SAH depolarization. More recently, our laboratory demonstrated that systemically administered cromakalim dose-dependently inhibits SAH-induced vasoconstriction in the rabbit basilar artery when treatment was initiated one hour after the hemorrhagic event. This finding suggests that cromakalim can block the induction of cerebral vasospasm, if treatment is started soon after SAH. Although the effect of cromakalim on other types of potassium channel openers cannot be ruled out entirely [21], the preceding results reinforce the idea that K_{ATP} channel openers may be of benefit in the treatment of cerebral vasospasm.

The potential clinical value of cromakalim for treating cerebral vasospasm will depend in part on its ability to reverse an established spastic response when administered systemically. The present study provides evidence that this may be possible. Intravenously administered cromakalim attenuated SAH-induced vascular constriction when treatment was initiated 24 hours after experimental hemorrhage. This effect was dose-dependent and highly significant. In the rabbit model of experimental SAH that was used in this study, the cross sectional area of the basilar is already constricted by approximately 32% at 24 hours post-SAH [8]. Consequently, treatment in the present study was given at a time when substantial constriction had

already been established in the vessels. It is important to note that the time course of post-SAH vasoconstriction in the rabbit SAH model is truncated relative to that of SAH-induced vasospasm in humans. Nonetheless, the rabbit model SAH-induced spasm does share key features with human vasospasm. Similar to human vasospasm, delayed vasoconstriction is observed in the rabbit after experimental SAH, and this constriction is relatively refractory to traditional vasodilators [12, 18]. The ability of cromakalim to reverse an established constrictor response in the rabbit SAH model raises the possibility that the more sustained form of vasospasm observed in humans may also be amenable to such treatment. An important step in the future evaluation of the therapeutic impact of cromakalim will be to verify its anti-spastic effects in a primate model of vasospasm, which exhibits a more prolonged form of cerebral vasospasm.

Conclusion

The present findings provide the first evidence that an intravenously-administered K^+ channel activator can attenuate SAH-induced vasospasm, even when treatment is initiated 1 day after SAH. The results also provide support for the concept that K_{ATP} channel openers represent a useful category of compounds for the therapeutic treatment of certain forms of cerebrovascular disease.

Acknowledgment

This work was supported in part by grant #HL49396.

References

1. Cain CR, Nicholson CD (1989) Comparison of the effects of cromakalim, a potassium conductance enhancer, and nimodipine, a calcium antagonist, on 5-hydroxytryptamine responses in a variety of vascular smooth muscle preparations. Naunyn-Schmiedebergs Arch Pharmacol 340: 293–299
2. Escande D, Thuringer D, Leguern S, Cavero I (1988) The potassium channel opener cromakalim (BRL34915) activates ATP-dependent K^+ channels in isolated cardiac myocytes. Biochem Biophys Res Commun 154: 620–625
3. Faraci MF, Heistad DD (1993) Role of ATP-sensitive potassium channels in the basilar artery. Am J Physiol 264: H8–13
4. Kassell NF, Peerless SJ, Drake CG, Boarini DJ, Adams HP (1980) Treatment of ischemic deficits from cerebral vasospasm with high dose barbiturate therapy. Neurosurgery 7: 593–597
5. Kassell NF, Drake CG (1983) Review of the management of saccular aneurysm. Neurol. Clin 1: 73–76
6. Kassell NF, Sasaki T, Colohan ART Nazar G (1985) Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Stroke 16: 562–572
7. Ksoll E, Parsons AA, Mackert JRL, Schilling L, Wahl M (1991) Analysis of cromakalim-, pinacidil-, and nicorandil-induced relaxation of the 5-hydroxytryptamine precontracted rat isolated basilar artery. Naunyn-Schmiedebergs Arch Pharmacol 343: 377–383
8. Kwan AL, Bavbek M, Jeng AY, Maniara W, Toyoda T, Lappe RW, Kassell NF, Lee KS (1997) Prevention and reversal of cerebral vasospasm by an endothelin-converting enzyme inhibitor, CGS 26303, in an experimental model of subarachnoid hemorrhage. J Neurosurg 87: 281–286
9. Kwan AL, Lin CL, Yanamoto H, Howng SL, Kassell NF, Lee KS (1998) Systemic administration of the potassium channel activator cromakalim attenuates cerebral vasospasm after experimental subarachnoid hemorrhage. Neurosurgery 42: 347–351
10. Masuzawa K, Asano M, Matsuda T, Imaizumi Y, Watanabe M (1990) Possible involvement of ATP-sensitive K^+ channels in the relaxant response of dog middle cerebral artery to cromakalim. J Pharmacol Exp Therap 225: 818–825
11. Nagao T, Sadoshima S, Kamouchi M, Fujishima M (1991) Cromakalim dilates rat cerebral arteries in Vitro. Stroke 22: 221–224
12. Nakagomi T, Kassell NF, Hongo K, Sasaki T (1990) Pharmacological reversibility of experimental cerebral vasospasm. Neurosurgery 27: 582–586
13. Nelson MT, Quayle JM (1995) Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol 268: C799–C822
14. Parsons AA, Ksoll E, Mackert JRL, Schilling L, Wahl M (1990) Effect of sulfonylureas on cromakalim induced relaxation of KCl precontracted rabbit, cat and rat isolated cerebral arteries. Br J Pharmacol Proc Suppl 100: 457
15. Parsons AA, Ksoll E, Mackert JRL, Schilling L, Wahl M (1991) Comparison of cromakalim-induced relaxation of potassium precontracted rabbit, cat, and rat isolated cerebral artery. Naunyn-Schmiedebergs Arch Pharmacol. 343: 384–392
16. Quayle JM, Mc Carron JG, Brayden JE, Nelson MT (1993) Inward rectifier K^+ currents in smooth muscle cells from rat resistance-sized cerebral arteries. Am J Physiol 265: C1363–C1370
17. Sanguinetti MC, Scott AL, Zingaro GJ, Siegl PK (1988) BRL 34915 (cromakalim) activates ATP-sensitive K^+ current in cardiac muscle. Proc Natl Acad Sci USA 85: 8360–8364
18. Sano K, Asano T, Tanishima T, Sasaki T (1980) Lipid peroxidation as a cause of cerebral vasospasm. Neurol Res 2: 253–272
19. Schilling L, Parsons AA, Wahl M (1995) Effects of potassium channel activators on isolated cerebral arteries of large and small diameter in the cat. J Neurosurg 83: 123–128
20. Standen NB, Quayle JM, Davies NW, Brayden JE, Huang Y, Nelson MT (1989) Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. Science 245: 177–180
21. Stockbridge N, Zhang H, Weir BK (1991) Effects of K^+ channel agonists cromakalim and pinacidil on rat basilar artery smooth muscle cells are mediated by Ca^{++}-activated K^+ channels. Biochem Biophys Res Com 181: 172–178
22. Yokoshiki H, Sunagawa M, Seki T, Sperelakis N (1998) ATP-sensitive K^+ Channels in pancreatic, cardiac, and vascular smooth muscle cells. Am J Physiol 274: C25–37
23. Zhang H, Stockbridge N, Weir B, Vollrath B, Cool D (1992) Vasodilatation of canine cerebral arteries by nicorandil, pinacidil and lemakalim. Gen Pharmacol 23: 197–201
24. Zhang H, Cook D (1994) Cerebral vascular smooth muscle potassium channels and their possible role in the management of vasospasm. Pharmacol Toxicol 75: 327–336

25. Zuccarello M, Rapoport RM (1993) Role of K^+ channels in vasospasm after subarachnoid hemorrhage. Stroke 24: 168
26. Zuccarello M, Bonasso CL, Lewis AI, Sperelakis N, Rapoport RM (1996) Relaxation of subarachnoid hemorrhage-induced spasm of rabbit basilar artery by the K^+ channel activator cromakalim. Stroke 27: 311–316

Comments

The objective of the presented manuscript was to analyse the effects of the K^+ channel activator Cromakalim on cerebral vasospasm after experimental subarachnoid hemorrhage, using a rabbit single-hemorrhage-model. Cromakalim was effective after intravenous administration elicting a dose-dependent reduction in SAH-induced vasospasm. In addition to the previously published data (Kwan *et al.* Neurosurgery 42: 347–351, 1998), Cromakalim led to a reduction of the spasm of the basilar artery, administerd after the vasoconstriction had already been initiated (24 hs.)

V. Seifert

Thank you very much for your letter concerning manuscript N° 1862 "Delayed administration of the K^+ Channel Activator Cromakalim Attenuates Cerebral Vasospasm after Experimental Subarachnoid Hemmorhage" by Kwan A L *et al.*

In a rather simple animal vasospasm model the authors proved that the K^+ channell activator cromakalim is capable of limiting constriction of the basilar artery not only via acute administration (i.e. before vessel constriction has been initiated) but also via delayed administration (i.e. after SAH-induced constriction has been initiated). The result confirms the working hypothesis that K_{ATP} channel activators could be of benefit for influencing SAH-induced vasospasm.

However, an ultimate clinical value of cromakalim for treating symptomatic vasospasm can be hoped only if it will also be effective in more complicated vasospasm models simulating human SAH-induced arterial vasoscontriction more appropriately (subarachnoid haemorrhage with raised ICP, etc.).

T. Doczi

Correspondence: Kevin S. Lee, Ph.D., University of Virginia, Department of Neuroscience, Box 5148 MR4 Building, Health Sciences Center, Charlottesville, Virginia 22908.