Laboratory study

The effect of caffeine on dilated cerebral circulation and on diagnostic CO_2 reactivity testing

Martin Blaha [a,b,*], Vladimir Benes [b], Colleen M. Douville [a,c], David W. Newell [a,c]

[a] Department of Neurological Surgery, School of Medicine, University of Washington, Seattle, Washington, United States
[b] Neurosurgery Department, Central Military Hospital, First Faculty of Medicine, Charles University, Prague, Czech Republic
[c] Seattle Neuroscience Institute, Swedish Medical Center/Providence Campus, Seattle, Washington, United States

Abstract

Reduction of cerebral blood flow by caffeine has been shown in multiple studies. However, the effect of this substance on pathologically dilated cerebral vessels is not clearly defined. The aim of this study was to investigate the effect of caffeine on an already dilated cerebral circulation and specify if these vessels are still able to constrict as a consequence of caffeine stimulation. A second aim of this study was to compare results of cerebral vasomotor CO_2 reactivity testing with and without caffeine ingestion. Seventeen healthy adult volunteers had vasomotor reactivity tested before and thirty minutes after ingestion of 300 mg of caffeine. Each vasomotor reactivity test consisted of velocity measurements from both middle cerebral arteries using transcranial Doppler ultrasound during normocapnia, hypercapnia, and hypocapnia. Hemodynamic data and end-tidal CO_2 ($etCO_2$) concentration were also recorded. The vasomotor reactivity (VMR) and CO_2 reactivity were calculated from a measured data pool. At a level of $etCO_2 = 40$ mmHg the resting velocity in the middle cerebral artery (V_{MCA}) dropped from 70.7 ± 22.8 cm/sec to 60.7 ± 15.4 cm/sec 30 minutes after caffeine stimulation (14.1% decrease, $p < 0.001$). During hypercapnia of $etCO_2 = 50$ mmHg there was also a significant decline of V_{MCA} from 103.1 ± 25.4 to 91.4 ± 21.8 cm/sec (11.3%, $p < 0.001$). There was not a statistically significant reduction of V_{MCA} during hypocapnia. Calculated VMR and CO_2 reactivity before and after caffeine intake were not statistically significant. The presented data demonstrate a significant decrease in cerebral blood flow velocities after caffeine ingestion both in a normal cerebrovascular bed and under conditions of peripheral cerebrovascular vasodilatation. These findings support the important role of caffeine in regulation of CBF under different pathological conditions. Despite significant reactive vasodilatation in the brain microcirculation, caffeine is still able to act as a competitive antagonist of CO_2 on cerebral microvessels. The fact that caffeine may decrease CBF despite significant pathological vasodilatation offers the possibility of therapeutic manipulation in patients with traumatic vasoparalysis. For routine clinical testing of CO_2 reactivity it is not necessary to insist on pre-test dietary restrictions.

1. Introduction

Methylxanthines are the most widely consumed central nervous system stimulants by man as components of coffee, tea, cola drinks or chocolate. Caffeine is the most commonly ingested methylxanthine.[1,2] Reduction of cerebral blood flow (CBF) by caffeine in healthy volunteers has been shown in multiple studies.[3–5] However, the effect of this substance on pathologically dilated cerebral vessels is not clearly defined. The aim of this study was to investigate the effect of caffeine on dilated cerebral circulation and to determine if these vessels are still able to constrict as a consequence of caffeine stimulation. This information may show the possible risk of caffeine-containing beverages for patients with decreased CBF and compensatory

* Corresponding author. Present address: Central Military Hospital, U Vojenske nemocnice 1200, 169 02 Prague – Stresovice, Czech Republic. Tel./fax: +420 973202963.

E-mail address: drmartinblaha@yahoo.com (M. Blaha).

vasodilatation. It could also be an important first step for pharmacological manipulation of traumatic vasoparalysis. A second aim of this study was to compare results of cerebral vasomotor CO_2 reactivity testing with and without caffeine ingestion. This test is used widely in the clinical setting as a diagnostic tool for different cerebrovascular diseases and the investigation of the effect of pre-test caffeine ingestion on the final results would be beneficial.

The potent effect of CO_2 on the cerebral microcirculation was used for dilatation of the cerebral vessels. The relative changes in CBF were monitored as changes in middle cerebral artery flow velocity, measured by transcranial Doppler ultrasound (TCD).

2. Subjects and methods

The study was approved by the University of Washington Human Subject Review Committee, and written informed consent was obtained from each volunteer.

2.1. Subjects

Seventeen healthy adult volunteers (7 male and 10 female) with no history of cerebrovascular diseases, participated in this study. Their ages were between 25 and 59 years (mean 39.9 ± 8.7). They were advised not to drink coffee or caffeine-containing beverages for 6 h prior to the test.

2.2. Hemodynamics measurement

A headband was placed on the subject's head, allowing a secure attachment of a left and right 2.0 mHz pulsed wave Doppler transducer over the temporal bone windows. Velocities (time averaged maximum or V_{mean}) from both middle cerebral arteries were continuously monitored at a depth providing the best signal (50–55 mm) using TCD equipment (MultidopX, DWL, Sipplingen, Germany) and standard vessel identification criteria. Non-invasive blood pressure and heart rate were recorded with a pneumatic cuff (CBM-7000, ColinCorp., San Antonio, TX, USA). End-tidal CO_2 concentration was measured with a CO_2 analyzer (Datex 223, Puritan-Bennett Corp., MA, USA).

2.3. CO_2 challenge

The subjects used a mouthpiece connected to a one-way non-rebreathing valve. After a resting period in a cardiac chair, we obtained baseline values while subjects were breathing room air. Thereafter, a CO_2 challenge test was performed by connecting the breathing system to a reservoir bag filled with 6% CO_2 and 94% oxygen. The subjects breathed gas enriched with CO_2 for 3 min and then the above-mentioned data was recorded. The reservoir bag was disconnected and while subjects were breathing room air they were asked to hyperventilate for 1 min and a recording of monitored values was again obtained. The

vasomotor reactivity (VMR) was calculated according to the following equation:

$$VMR = (V_{mean-hypercapnia} \times 100/V_{mean-normocapnia})$$
$$- (V_{mean-hypocapnia} \times 100/V_{mean-normocapnia})$$

CO_2 reactivity (% change in velocity per mmHg CO_2) was calculated as:

$$CO_2 \text{ reactivity} = VMR/(CO_{2\,maximum} - CO_{2\,minimum})$$

2.4. Testing protocol

The vasomotor reactivity was measured for each subject. Thereafter, the subject was asked to drink 350 mL of drip coffee, which contained 300 mg of caffeine. A repeat measurement of vasomotor reactivity was done 30 minutes after coffee ingestion. The dose of caffeine in drip coffee was measured by high pressure liquid chromatography (HPLC).

2.5. Statistical analysis

Data were reported as mean $\pm$ standard deviation (SD) and the changes in velocity in the middle cerebral artery (V_{MCA}) were calculated as differences from the baseline values. Data evaluation was carried out by standard techniques (paired t-test) and a value of $p < 0.05$ was assumed to be significant.

3. Results

Results are summarized in Table 1. The baseline V_{MCA} during normocapnic conditions was 73.6 ± 24.9 cm/sec. Thirty minutes after caffeine intake V_{MCA} dropped to 58.9 ± 17.1 cm/sec, which represents a 20.1% decrease. As CO_2 is a powerful CBF regulator, it was necessary to rule out its influence on data interpretation. The baseline velocities were measured during end-tidal CO_2 (etCO_2) at 41.2 ± 3.1 mmHg and again after

Table 1
Results

	Units of measure	Baseline	After caffeine
etCO$_2$-normocapnia	mmHg	41.2 ± 3.1	38.8 ± 3.1
V$_{MCA}$	cm/s	73.6 ± 24.9	58.9 ± 17.1
V$_{MCA}$-corrected for etCO$_2$ = 40 mmHg	cm/s	70.7 ± 22.8	60.7 ± 15.4
etCO$_2$-hypercapnia	mmHg	50.9 ± 3.2	50.1 ± 2.7
V$_{MCA}$-hypercapnia	cm/s	104.7 ± 25.6	91.7 ± 23.0
V$_{MCA}$-corrected for etCO$_2$ = 50 mmHg	cm/s	103.1 ± 25.4	91.4 ± 21.8
etCO$_2$-hypocapnia	mmHg	22.2 ± 2.8	20.9 ± 2.0
V$_{MCA}$-hypocapnia	cm/s	42.8 ± 11.9	38.9 ± 9.4
V$_{MCA}$-corrected for etCO$_2$ = 20 mmHg	cm/s	38.2 ± 12.2	37.6 ± 10.5

etCO$_2$, end-tidal CO$_2$; V$_{MCA}$, velocity in the middle cerebral artery.

caffeine intake with resting $etCO_2$ at 38.8 ± 3.1 mmHg (significant difference, $p < 0.05$). We recalculated data from every volunteer on a theoretical level of $etCO_2 = 40$ mmHg using the final data from VMR testing minus the percentage change of V_{MCA} per 1 mmHg of $etCO_2$. Under these conditions, the resting V_{MCA} dropped from 70.7 ± 22.8 cm/sec to 60.7 ± 15.4 cm/sec after caffeine stimulation. This represents a 14.1% decrease from baseline values. Both results, with and without CO_2 adjustment, showed a statistically significant reduction of CBF after caffeine intake ($p < 0.001$). The adjustment for CO_2 influence was also used for data acquired during hyper-and hypocapnia.

The baseline measurements during hypercapnia resulted in an $etCO_2 = 50.9 \pm 3.2$ mmHg and V_{MCA} of 104.7 ± 25.6 cm/sec, with a reduction of 12.4% after caffeine intake to a V_{MCA} of 91.7 ± 23.0 cm/sec ($etCO_2 = 50.1 \pm 2.7$ mmHg). Recalculated data at an $etCO_2$ of 50 mmHg showed a decline from 103.1 ± 25.4 to 91.4 ± 21.8 cm/sec (11.3%). Both results reached statistical significance ($p < 0.001$). Finally, the baseline mean velocities during hypocapnia measured at $etCO_2 = 22.2 \pm 2.8$ were 42.8 ± 11.9 cm/sec, and after caffeine stimulation the average $etCO_2$ dropped to 20.9 ± 2.0 and V_{MCA} was 38.9 ± 9.4 cm/sec. Adjusted data on $etCO_2$ level of 20 mmHg showed only a minor decrease from 38.2 ± 12.2 cm/sec to 37.6 ± 10.5 cm/sec. Although the first dataset showed a statistical significance of $p < 0.05$, the recalculated data did not prove a statistically significant reduction of V_{MCA} during hypocapnia.

The hemodynamic parameters during experiments are shown in Table 2. Despite the elevation in both systolic and diastolic pressures; only the increase in systolic pressure is statistically significant ($p < 0.05$).

Calculated baseline VMR and CO_2 reactivity were 86.5 ± 19.1 and $3.03 \pm 0.6\%$/mmHg respectively (Table 3). After caffeine intake they reached 90.6 ± 3.1 and $3.1 \pm 0.6\%$/mmHg. These changes were not statistically significant ($p = 0.197$ and $p = 0.354$, respectively).

Table 2
Results – hemodynamic parameters during experiments

	Baseline	After caffeine
SBP (mmHg)	113.6 ± 11.7	118.1 ± 9.6
DBP (mmHg)	64.8 ± 7.9	67.2 ± 8.9
HR (beats/min)	70 ± 12.4	65.7 ± 11.3
$etCO_2$ (mmHg)	41.2 ± 3.1	38.8 ± 3.1

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; $etCO_2$, end-tidal CO_2.

Table 3
Results – VMR, CO_2 reactivity

	Baseline	After caffeine
VMR	86.5 ± 19.1	90.6 ± 13.7
CO_2 reactivity (mmHg)	3.0 ± 0.6	3.1 ± 0.6

VMR, vasomotor reactivity.

4. Discussion

Adenosine is identified as a potent cerebral vasodilator and has been proposed as a metabolic regulator of CBF. Adenosine-induced vasodilatation in cerebral microvessels involves cGMP and cGMP-dependent protein kinase.[6] The receptor subtypes that mediate the dilatation of vessels have been investigated in the rat brain and showed the A_{2A} receptor subtype mediates dilatation of cerebral arterioles to adenosine, and the A_{2B} receptor sybtype is probably also involved in adenosine-induced dilatation, while there is evidence against the involvement of both A_1 and A_3 receptors.[7] Caffeine, which is the most widely consumed neural stimulant in the world, with most of the intake coming from dietary sources such as coffee, tea, chocolate and soft drinks,[1,2] acts as an adenosine receptor antagonist and reduces CBF primarily by inhibition of the A_{2A} receptors.[8,9] Meno and colleagues showed that caffeine in rats attenuated both resting diameter and CBF responses to both adenosine and to somatosensory stimulation and they proposed a role of adenosine in the regulation of CBF during functional neuronal activity.[8] Interestingly, hypercapnic vasodilatation was unaffected by caffeine.[8]

There are two methodological considerations. The first is the reliability of TCD to measure relative changes in CBF. As Serrador et al. and Newell et al. observed, there is no change in the MCA diameter during different CO_2 levels and during hypotension.[10,11] So we can conclude that relative changes in MCA flow velocities are representative of changes in CBF. The second issue arises from the simulation of pathological vasodilatation. Hypercapnia does not simulate pathophysiological vasodilatation, therefore the experimental data should be analysed as results of hypercapnic conditions with possible similarities for other events.

The presented data demonstrated a significant decrease in CBF after caffeine ingestion both in a normal cerebrovascular bed and under conditions of cerebrovascular vasodilation. These findings support the important role of caffeine in regulation of CBF under different pathological conditions. Despite significant reactive vasodilatation in the brain microcirculation, the caffeine is still able to work as a competitive antagonist of CO_2 on cerebral microvessels. The presumed mechanism of action is through the blockage of A_{2A} and A_{2B} receptors.[7] Further research into caffeine effects on cerebral vessels may offer new therapeutic approaches and might bring new methods of CBF manipulation. Recently, it has been reported that the administration of caffeinol (caffeine plus ethanol) provides distinct neuroprotection in an animal model of transient common carotid/MCA occlusion. Caffeinol reduced cortical infarct volume loss up to 80% and improved motor coordination.[12–14] A pilot study in humans (23 stroke patients) explored the safety of caffeinol alone or combined with intravenous tissue plasminogen activators.[15] It has also been demonstrated that early post-traumatic administration of caffeinol reduces contusion volume and improves working memory in rats.[16]

The fact that caffeine may decrease CBF despite significant pathological vasodilatation offers the possibility of therapeutic manipulation of traumatic vasoparalysis. We suggest caffeine may decrease intracranial pressure similar to hypocapnia. To explore this hypothesis an animal model study is needed.

The importance of diet prerequisites for diagnostic CO_2 reactivity testing is questionable. Despite different values there was no statistical distinction in results with or without caffeine. We would advise routine clinical testing avoiding dietary restrictions. However, in research it would be preferable to have dietary limitations to obtain comparable data.

Acknowledgments and Funding

References

1. Galasko GT, Furman KI, Alberts E. The caffeine contents of non-alcoholic beverages. *Food Chem Toxicol* 1989;**27**:49–51.
2. Nehlig A, Daval JL, Debry G. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psycho-stimulant effects. *Brain Res Rev* 1992;**17**:139–70.
3. Mathew RJ, Wilson WH. Caffeine induced changes in cerebral circulation. *Stroke* 1985;**16**:814–7.
4. Cameron OG, Modell JG, Hariharan M. Caffeine and human cerebral blood flow: a positron emission tomography study. *Life Sci* 1990;**47**:1141–6.
5. Mathew RJ, Barr DL, Weinman ML. Caffeine and cerebral blood flow. *Br J Psychiatry* 1983;**143**:604–8.
6. West GA, Meno JR, Nguyen TS, et al. cGMP-dependent and not cAMP-dependent kinase is required for adenosine-induced dilation of intracerebral arterioles. *J Cardiovasc Pharmacol* 2003;**41**:444–51.
7. Ngai AC, Coyne EF, Meno JR, et al. Receptor subtypes mediating adenosine-induced dilation of cerebral arterioles. *Am J Physiol Heart Circ Physiol* 2001;**280**:2329–35.
8. Meno JR, Nguyen TS, Jensen EM, et al. Effect of caffeine on cerebral blood flow response to somatosensory stimulation. *J Cereb Blood Flow Metab* 2005;**25**:775–84.
9. Mathew RJ, Wilson WH. Substance abuse and cerebral blood flow. *Am J Psychiatry* 1991;**148**:292–305.
10. Serrador JM, Picot PA, Rutt BK, et al. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* 2000;**31**:1672–8.
11. Newell DW, Aaslid R, Lam A, et al. Comparison of flow and velocity during dynamic autoregulation testing in humans. *Stroke* 1994;**25**:793–7.
12. Aronowski J, Strong R, Shirzadi A, et al. Ethanol plus caffeine (caffeinol) for treatment of ischemic stroke: preclinical experience. *Stroke* 2003;**34**:1246–51.
13. Strong R, Grotta JC, Aronowski J. Combination of low dose ethanol and caffeine protects brain from damage produced by focal ischemia in rats. *Neuropharmacology* 2000;**39**:515–22.
14. Zhao X, Liu SJ, Zhang J, et al. Combining insulin-like growth factor derivatives plus caffeinol produces robust neuroprotection after stroke in rats. *Stroke* 2005;**36**:129–34.
15. Piriyawat P, Labiche LA, Burgin WS, et al. Pilot dose escalation study of caffeine plus ethanol (caffeinol) in acute ischemic stroke. *Stroke* 2003;**34**:1242–5.
16. Dash PK, Moore AN, Moody MR, et al. Post-trauma administration of caffeine plus ethanol reduces contusion volume and improves working memory in rats. *J Neurotrauma* 2004;**21**:1573–83.