Ischemic stroke increases heart vulnerability to ischemia-reperfusion and alters myocardial cardioprotective pathways

Alexandre Meloux, Eve Rigal, Luc Rochette, Yves Cottin, Yannick Bejot, Catherine Vergely

Ischemic stroke increases heart vulnerability to ischemia-reperfusion and alters myocardial cardioprotective pathways.

Alexandre Meloux[1,2], MSc; Eve Rigal[1], MSc; Luc Rochette[1], PhD, PharmD; Yves Cottin[1,2], MD, PhD; Yannick Bejot[1,3], MD, PhD; Catherine Vergely[1]*, PhD, PharmD.

[1] Equipe d'Accueil (EA 7460): Physiopathologie et Epidémiologie Cérébro-Cardiovasculaires (PEC2) Université de Bourgogne-Franche-Comté; UFR des Sciences de Santé; 7 Bd Jeanne d'Arc, 21000 Dijon - France

[2] Department of Cardiology - University Hospital of Dijon, France

[3] Department of Neurology - University Hospital of Dijon, France

*Address correspondence to this author: Tel: +33-380393460; Fax: +33-380393293

E-mail address: catherine.vergely@u-bourgogne.fr

Cover title: Ischemic stroke and myocardial dysfunction

Total number of figures: 6

Key words: Brain, Heart, Stroke, Myocardial ischemia, Nitro-oxidative stress, Autonomic nervous system

Subject terms: Ischemia, Myocardial Infarction, Ischemic Stroke

Word count: 5,498 words

Abstract

Background and Purpose - For years, the relationship between cardiac and neurological ischemic events has been limited to overlapping pathophysiological mechanisms and common risk factors. However, acute stroke may induce dramatic changes in cardiovascular function. The aim of this study was to evaluate how prior cerebrovascular lesions affect myocardial function and signaling *in vivo* and *ex vivo*, and how they influence cardiac vulnerability to ischemia-reperfusion (IR) injury.

Methods - Cerebral embolization was performed in adult Wistar male rats through the injection of microspheres into the left or right internal carotid artery. Stroke lesions were evaluated by microsphere counting, tissue staining and assessment of neurological deficit 2h, 24h and 7 days after surgery. Cardiac function was evaluated *in vivo* by echocardiography and *ex vivo* in isolated perfused hearts. Heart vulnerability to IR injury was investigated *ex vivo* at different times post-embolization and with varying degrees of myocardial ischemia. Left ventricles (LV) were analyzed with western blotting and quantitative RT-PCR.

Results – Our stroke model produced large cerebral infarcts with severe neurological deficit. Cardiac contractile dysfunction was observed with an early but persistent reduction of LV fractional shortening *in vivo* and of LV developed pressure (LVDevP) *ex vivo.* Moreover, after 20 or 30 minutes of global cardiac ischemia, recovery of contractile function was poorer with impaired LVDevP and relaxation during reperfusion in both stroke groups. Following stroke, circulating levels of catecholamines and GDF15 increased. Cerebral embolization altered nitro-oxidative stress signaling and impaired the myocardial expression of ADRB1 and cardioprotective SAFE signaling pathways.

Conclusions - Our findings indicate that stroke not only impairs cardiac contractility and also worsens myocardial vulnerability to ischemia. The underlying molecular mechanisms of

stroke-induced myocardial alterations after cerebral embolization remain to be established, insofar as they may involve the sympathetic nervous system and nitro-oxidative stress.

Introduction

Cardiovascular and cerebrovascular diseases share a number of pathophysiological mechanisms and risk factors, and they remain among the leading causes of death and medical spending worldwide. While it is well documented that cardiac diseases such as atrial fibrillation[1, 2] or acute myocardial infarction[3] (AMI) may favor the occurrence of ischemic stroke (IS), some clinical data also suggest that acute cerebral ischemic events may in turn induce dramatic shifts in cardiovascular function[4]. For instance, Takotsubo cardiomyopathy may occur in the days following an IS[5]. Furthermore, it has been shown that ischemic lesions in specific brain regions such as the insular cortex are associated with cardiac dysfunction and sudden cardiac death[6]. Indeed, even in the absence of documented preliminary heart disease, myocardial dysfunction is commonly encountered in stroke patients [7-10]. Cardiovascular complications after stroke are the second cause of death after the direct consequences of neurological damage[11, 12]. Moreover, a significant proportion of patients with ischemic stroke display elevated levels of cardiac troponins[7, 13, 14], relevant markers of myocardial damage, though half of them show no angiographic evidence of coronary artery disease[15]. Therefore, the possibility of stroke-induced myocardial dysfunction/injury needs to be investigated more thoroughly.

In experimental studies, a link has been established between brain damage and cardiac disorders. For instance, several studies have shown that middle cerebral artery occlusion (MCAO) could impair calcium and other ionic currents in ventricular cardiomyocytes, thus inducing arrhythmia in rats[16, 17], and may also lead to cardiac contractile dysfunction in mice[18]. Another study further suggests that ischemic brain tissues send “death signals” to the heart[19]. Recently, it has been proposed that circulating components might directly be involved in cerebral ischemia-induced cardiac dysfunction such as MiR-126[20] or catecholamines[21].

However, to date, no work has extensively explored the potential vulnerability to myocardial ischemia/infarction associated with prior ischemic stroke.

Therefore, it seems essential to evaluate how prior cerebral ischemia could alter cardiac function and its subsequent adaptation to ischemic stress, and which cardiac cellular signaling pathways involved in cardioprotection are impaired. The aim of this study was thus to mimic a situation of embolic stroke and to evaluate how cerebrovascular lesions may affect the heart functions both at the *in vivo*, *ex vivo* and cellular level.

Methods

This article adheres to the AHA Journals implementation of the Transparency and Openness Promotion Guidelines. Details of materials and methods are available in the Data Supplement. Data that support the findings of this study are available from the corresponding author on reasonable request.

Animals

Wistar male rats (Charles River, Wilmington, USA) (8-9 weeks, 300±50 g) were used. All animals received humane care and study protocols complied with institutional guidelines. The investigation was carried out in accordance with Directive 2010/63/EU of the European Parliament and to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the local ethics committee (Comité d'Ethique de l'Expérimentation Animale Université-Bourgogne-Franche-Comté, Dijon, France, protocol agreement number: 0412.01). The animals were housed at 21±2°C with a constant humidity of 55±10% under a cycle of 12 h of light/dark and had free access to water and standard diet *ad libitum*.

Model of cerebral embolization

Polystyrene microspheres (90 μm; Polysciences, Inc., Warrington, USA) were used for cerebral embolization. The solution was diluted so as to inject around 4,000 microspheres in 200 μL of 20% polyvinylpyrrolidone (PVP) in the internal carotid artery. A detailed description of surgical procedure is available in the supplemental data.

Neurological deficit assessment and determination of cerebral embolization and infarct size

Neurological deficit was assessed 2 h, 24 h or 7 days after surgery using a modified scoring system developed by Longa et al.[22] by an observer blinded to the animal groups. Deficits were

scored as follows: 0, no deficits; 1, failure to extend contralateral forepaw fully; 2, circling to contralateral side without falling; 3, falling to contralateral side; 4, no spontaneous motor activity. Higher scores represent more severe motion impairment. A detailed description of cerebral embolization determination and infarct size measurement are available in the supplemental data.

Assessment of cardiac function in vivo with echocardiography

Transthoracic echocardiography using the Vevo770™ imaging system (VisualSonics Inc., Toronto, Canada), equipped with a 25 MHz probe was performed 7 days before surgery, 2 h, 24 h and 7 days after surgery in rats as previously described[23] and detailed in the supplemental data.

Assessment of cardiac function ex vivo

Two or 24 hours after cerebral embolization, rats were deeply anaesthetized through an intraperitoneal injection of 110 mg/kg sodium pentobarbital and the blood clotting was avoided by the co-administration of 500 IU/kg of heparin. After total loss of nociception, the chest was opened and the heart was excised and placed in a cold (4°C) perfusion buffer bath until contractions ceased. The heart was then immediately cannulated through the aorta and perfused according to the Langendorff method as previously described[24] and detailed in the supplemental data.

Measurement of myocardial vulnerability to ischemia-reperfusion injury ex vivo

After baseline measurements, perfusion was turned off so as to induce 20 or 30 minutes of total global normothermic ischemia, which was followed by 1 h of reperfusion. All parameters were recorded during ischemia and reperfusion.

Determination of plasma Epinephrine, Norepinephrine and Growth Differentiation Factor 15 (GDF15)

Blood was collected from the abdominal aorta, and immediately centrifuged at 4°C in order to separate the plasma, and samples were stored at -80°C. Plasma epinephrine (E) and norepinephrine (NE) levels were measured using the 2-CAT (A-N) Research ELISA kit (BA E-5400; Labor Diagnostika Nord, Nordhorn, Germany) following the manufacturer's protocol. Plasma GDF15 was measured using a commercially available kit (MGD150, R&D systems, Minneapolis, USA).

Quantitative Real-Time Polymerase Chain Reaction

Semiquantitative analysis of endothelial nitric oxide synthase (eNOS), glutathione peroxidase 1 (GPX1), catalase (CAT), adrenoceptor β1 (ADRB1), janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3) were performed and procedure is detailed in the supplemental data.

Western blotting

Ventricular tissue was homogenized in 10 volumes radio-immunoprecipitation assay (RIPA) buffer, centrifuged at 10,000 g for 15 min at 4°C and determination of protein concentration (Lowry method) in supernatant. Protein expressions were evaluated by Western blotting, and detailed procedures are available in the supplemental data.

Statistics

Data are presented as mean±SEM. The statistically significant differences for mean values of three groups were tested by analysis of variance (ANOVA) followed by Tukey's test. An ANOVA for repeated measurements followed by a Tukey post-hoc analysis was applied for

data consisting of repeated observations at successive time points. The difference was considered significant when $P < 0.05$. The data were analyzed using SigmaPlot version 12.5.

Results

Cerebral embolization with microspheres leads to large stoke infarcts and neurological deficit (supplemental data, Figure I)

Severe neurological deficit scores were observed in all stroke groups 2 h, 24 h and 7 days after embolization. Two hours after embolization, 397±33 microspheres were found in the ipsilateral hemisphere and 54±5 in the contralateral hemisphere. TTC staining was performed in brain slices 24 h after embolization. Sham-operated rats showed uniform red coloration of viable grey matter cell-containing areas, and white staining of acellular white matter fibers (*corpus callosum*, internal capsule). In embolized rats, white necrotic areas were only observed in the ipsilateral hemisphere. Stroke volumes were 31±4% for the left ventricle and 29±4% for the right ventricle 24 h after embolization.

Cerebral embolization leads to cardiac dysfunction ex vivo and in vivo

Cardiovascular function was initially assessed *in vivo*. There were no statistically significant differences between the three groups concerning arterial systolic blood pressure (Figure 1a). We found an early (from H2) and persistent (until D7) deterioration in LV fractional shortening over the 7-day follow-up period in both stroke versus sham groups (Figure 1b).

Then, in order to evaluate post-stroke cardiac function independently of any circulating and/or nervous influencing factors, we isolated and perfused the rat hearts *ex vivo* and measured left ventricular functional parameters. Two hours after cerebral embolization, the baseline LVDevP was significantly lower in both groups of stroke rats than in the sham group: 106.6±4.4 mmHg [left], 106.2±4.7 mmHg [right] *vs.* 129.9±2.7 mmHg [sham] (Figure 1c). This difference was also observed 24 h after surgery: 91.8±3.7 mmHg [left], 104.0±5.3

mmHg [right] *vs* 120.9±5.1 mmHg [sham] (Figure 1d). The other functional parameters were similar for the three groups during basal perfusion.

Cerebral embolization increases cardiac vulnerability to ischemia-reperfusion injury ex vivo

In order to evaluate how prior ischemic stroke may influence myocardial vulnerability to ischemia-reperfusion injury, we exposed hearts isolated 2 hours after cerebral embolization to 30 minutes of global total ischemia, which is generally considered as partially irreversible. Reperfusion led to only limited recovery of the initial cardiac functional parameters. In the right stroke group, the post-ischemic recovery of coronary flow (CF) was significantly lower than that in the sham group: 8.3±0.1 mL/min *vs* 10.6±0.2 mL/min respectively, $p<0.05$ (Figure 2a). During ischemia, the heart stopped beating, but an increase in LV diastolic pressure due to ischemic contracture was observed in both groups. Reperfusion led to an abrupt and sharp increase in LV diastolic pressure, related to post-ischemic contracture that was even higher in the right stroke group than that in the sham group from the 20^{th} min of reperfusion: 90.6±4.2 mmHg *vs* 72.6±4.3 mmHg respectively, $p<0.05$ (Figure 2b). After 30 min of global total ischemia, reperfusion led to only a partial recovery of myocardial contractility in all groups, with identical return to an altered sinus rhythm (data not shown). However, the LVDevP and -dP/dt were significantly lower in both right and left stroke groups than in the sham group after 60 min of reperfusion (Figure 2c and d).

In a second set of experiments, we evaluated the myocardial vulnerability to a more reversible ischemia-reperfusion injury, and subjected isolated hearts to 20 minutes of global total ischemia followed by reperfusion. In these conditions, all three groups recovered total CF with hyperemia during the 20 first minutes of reperfusion (Figure 3a). However, concerning cardiac contractility, HR, LVDevP and -dP/dt recovered significantly more slowly during reperfusion in both stroke groups than in the sham group (Figure 3b, c and d).

Myocardial recovery after ischemia-reperfusion injury was also evaluated 24 h after stroke or sham embolization (Figure II in the online-only Data Supplement). However, unattended nearly total recovery of all functional parameters was observed in sham groups, as compared to the same duration of cardiac ischemia in hearts harvested 2 h after microsphere embolization or sham-surgery under isoflurane anesthesia. In these very different conditions, post-ischemic recovery of myocardial functional parameters did not differ between groups, except for HR or LVDevP in left stroke hearts at certain points in time.

Cardiac dysfunction after cerebral embolization is associated with increased catecholamine and GDF15 levels in circulation

In order to evaluate the activation of the autonomic nervous system, we assessed plasma concentration of catecholamines. At 2 h and 24 h after embolization, both stroke groups showed a significant increase in plasma E and NE as compared to sham operated rats (Figure 4a and b).

GDF15, a marker of several conditions including cardiovascular disease, was circulating at higher levels 2 h after cerebral embolization in both groups of stroke rats than in the sham group (Figure 4c): 185.3±13.3 ng/L [left], 137.0±10.4 ng/L [right] *vs.* 96.7±4.3 ng/L [sham]. This difference was also observed 24 h after surgery: 203.9±20.5 ng/L [left], 157.4±7.6 ng/L [right] *vs.* 113.0±8.3 ng/L [sham] (Figure 4d).

Cerebral embolization alters nitro-oxidative signaling in the heart.

To examine the potential impact of stroke on myocardial cellular signaling, genes and proteins involved in nitro-oxidative stress were assed in the myocardium. Left or right cerebral embolization significantly increased the cardiac expression of eNOS and GPX1 in mRNA and protein and decreased protein expression of peNOS. CAT protein expression was

decreased in the hearts of left or right stroke rats without modifications of the mRNA levels (Figure 5 and Figure III in the online-only Data Supplement).

Cerebral embolization impaired myocardial expression of ADRB1 and cardioprotective SAFE signaling pathway.

The expression of the main cardiac adrenergic receptor, ADRB1, was investigated in order to evaluate the potential mechanism of stroke-induced myocardial contractile dysfunction, despite increased circulating epinephrine and norepinephrine levels. The results showed decreased protein expression of ADRB1 (Figure 6 A).

So as to understand how prior cerebral infarction was able to worsen myocardial functional recovery after either reversible or irreversible cardiac ischemic injury, members of the SAFE cardioprotective signaling pathway were assessed in the myocardium. The results showed that left or right cerebral embolization significantly decreased short-term cardiac expression of JAK2, STAT3 and pSTAT3 in protein (Figure 6).

Discussion

In the present study we show for the first time that prior cerebral ischemia in rats can increase myocardial vulnerability to ischemia-reperfusion injury and alter cellular signaling in the genes and proteins generally considered as providing cardioprotection, such as endothelial nitric oxide synthase and members of the SAFE pathway. We have also highlighted a change in cardiac contractile function both *in vivo* and *ex vivo*, the latter being observed in a situation where neither circulating factors nor nervous control could influence cardiac function, suggesting that stroke has a persisting imprinting effect on heart tissue. Sympathetic hyperactivity with a rise of circulating catecholamines, as opposed to a down-regulation of cardiac ADRB1 protein could be considered as one of the potential mechanisms of stroke-driven cardiovascular deleterious effects.

The model of cerebral embolization with microsphere injection into the internal carotid artery was chosen since it may reproduce some aspects of embolic strokes of cardiac or carotid origin[25]. One limitation of this model is that microspheres are randomly distributed throughout the brain and produce disseminated infarcts, as opposed to conventional MCAO for which stroke volume and location in specific areas of the brain can be evaluated. In fact, it could be interesting to induce damage in defined brain regions such as the insular cortex, the activation of which leads to cardiovascular consequences[6]. Finally, the microsphere embolization model induces non-reversible artery occlusion and produces the creation of small permanent cerebral infarcts[26], while in stroke patients reperfusion can occur spontaneously or be clinically induced by thrombolysis. However, the majority of patients with large vessel stroke do not have recanalization[27]; our model may therefore be appropriate for preclinical evaluation of large vessel occlusion.

Brain-heart interactions after stroke have been observed in several clinical and animal studies, and recent data further suggest that aging, hypertension and diabetes contribute to distinctly affect microvessels gene network in these two organs[28]. Indeed, atrial fibrillation may occur after an IS[29]: in the 3 months following an IS, 19% of patients experience serious cardiac adverse events[12] and 6.5% of patients develop AMI or acute heart failure[30]. From an experimental point of view, *in vivo* studies have shown that cerebral injuries can induce severe cardiac arrhythmia, myocardial dysfunction, and even cardiomyocyte necrosis[18, 31]. Experiments performed *ex vivo* have shown that cardiomyocytes isolated from the hearts of right MCAO rats displayed longer action potential duration[17, 31]. In our study, IS involved the insular cortex which may have had an impact on cardiac function *in vivo* (see Figure 2). One of the striking observations of our work is that prior IS was not only able to decrease LV contractility *in vivo*, but also *ex vivo* in isolated perfused hearts, where neither circulating nor nervous factors influence cardio-vascular function. This observation is remarkable because it suggests that cerebral ischemia has a persistent impact on the heart through signals that affect its function in addition to direct endocrine and/or neuronal effect, and that this retained effect occurs as early as 2 hours after embolization. Our findings therefore corroborate other studies showing that MCAO in rats was able to impact the calcium currents and channels of the cardiomyocyte, as well as other ionic currents and proteins involved in the action potential[17, 31] or in the cardiac excitation-contraction coupling[16].

Another original result of the present study is that prior cerebral ischemia is able to increase myocardial vulnerability to ischemia-reperfusion injury *ex vivo*. Indeed, we found a negative impact of cerebral embolization on functional recovery of isolated hearts after either reversible or irreversible total global ischemia. The deleterious effect of stroke is clear 2 hours after surgery, but is partially hidden after a longer lapse of time (24 h), probably because of the delayed preconditioning effect of both isoflurane anesthesia and the surgical procedure.

The observation that prior cerebral ischemia may worsen myocardial post-ischemic recovery can be regarded as paradoxical in view of the well-known concept of remote ischemic conditioning, by which ischemia to a distant organ may protect the heart against acute myocardial infarction. When extended to the clinical situation, these results may raise the question of whether stroke patients have increased vulnerability to acute myocardial infarction. Our results may therefore corroborate data showing that patients who developed an AMI after an IS had an increased mortality rate versus the general popuation[30]. We also observed that right hemispheric strokes were more damaging for cardiac function and post-ischemic recovery than left hemispheric strokes. In fact, it has been shown both in humans and in experimental studies that a lateralization exists concerning cardiac effects of cerebral ischemia, which is related to that distinct areas in the left or right brain insula control either the sympathetic or parasympathetic autonomic nervous system[32]. Indeed, several studies have shown that right stroke begets more cardiovascular effects such as arrhythmias, or increased myocardial damage confirmed by elevated troponin levels[21, 31, 33]. It has been proposed that right-sided insular cortex regulates sympathetic tone whereas left controls parasympathetic. Vascular damage in right hemisphere involves a downregulation of parasympathetic activity with an upregulation of the sympathetic consequence on cardiac function[33]. However, the microsphere embolization model used in the present study, as stated before, does not allow for a study of the impact of revascularization on stroke-induced cardiac effects. Future studies with a model allowing both reperfusion, damage in selective brain regions, and different stroke durations such as the MCAO model are planned, so as to determine whether the myocardial ischemic vulnerability following an IS persists when the brain is reperfused and if the cardiac impact of cerebral ischemia is still present at a later time after stroke.

Following these observations, we aimed to determine which cardiac cellular pathways could be modified by prior stroke, and thus could be involved in these stroke-induced alterations of

myocardial function. It is well known that stroke is associated with an activation of the sympathetic nervous system, which is consistent with our data showing increased catecholamine levels in stroke groups[18, 21]. It has been shown that beta1-blocker treatment in stroke mice improved cardiac function and decreased catecholamine levels 2 months after IS and had neuroprotective effects over the 7 day follow-up period[21, 34]. In the present study, a down-regulation of ADRB1 was observed in hearts 2 h after embolization, which could be a result of post-translational regulating systems. GDF15 levels were also increased in stroke rats 2 h and 24 h after embolization as compared to sham groups. It has been shown that GDF15 levels were significantly increased in patients with ischemic stroke and even more when stroke involved large arteries. Indeed, GDF15 gene polymorphism may play a role in the development of ischemic stroke[35]. Concomitantly to ischemic stroke, several neurohumoral systems, including inflammatory cytokines, are activated, and recent data have shown that chronic inflammation may propagate from the ischemic cerebellum to distant peripheral organs such as the heart[36]. The occurrence of a nitro-oxidative stress could also be involved in these brain-heart interactions, which has been shown after transient MCAO in rats[37]. The increased gene and protein expression of GPX1 should be opposed to decrease in CAT which has an eight-fold higher activity than GPX1 in humans[38]. Additionally, it has been shown that some cardioprotective molecular pathways could be recruited by the cardiac tissue in order to fight the damaging effects of ischemia reperfusion injury[39]. Taking into consideration the idea that prior stroke impaired post-ischemic cardiac recovery, we hypothesized that some members of the cardioprotective pathways might have been altered after cerebral ischemia. In fact, JAK2, STAT3 and pSTAT3 protein expression, all members of the Survivor Activating Factor Enhancement (SAFE) cardioprotective signaling pathway, decreased in the heart 2 h after cerebral embolization. Therefore, the increased vulnerability of the heart to ischemia reperfusion injury after stroke might be, at least in part, related to an impairment of the SAFE

signaling pathway. An up-regulation or a pharmacological induction of these signaling pathways could therefore be used as therapeutic target to protect the heart after ischemic stroke.

Conclusions

These original data indicate not only that, in a model of cerebral embolization in rats, stroke deleteriously impairs cardiac contractility but also worsens myocardial vulnerability to ischemia. At the molecular level, we highlighted sympathetic hyperactivity, a disturbance of nitro-oxidative regulators in the heart, and impairment of the cardioprotective SAFE signaling pathway. The underlying molecular mechanisms of the stroke-induced myocardial alterations after cerebral embolization remain to be established, insofar as they may involve the sympathetic nervous system or and nitro-oxidative stress. From a clinical point of view, these results may be of major importance for the management of stroke patients, in order to protect the heart after cerebral ischemia.

Disclosures

Yannick Béjot : honoraria for lectures or consulting fees from AstraZeneca, Daiichi-Sankyo, BMS, Pfizer, Medtronic, MSD France, Amgen, and Boehringer-Ingelheim.

Alexandre Méloux, Luc Rochette, Yves Cottin, Eve Rigal, Catherine Vergely: None

Acknowledgements

The authors thank Suzanne Rankin for English revision of the manuscript, Ivan Porcherot and Angeline Jobez for technical assistance.

Funding sources

This study has been supported by funding from the French Ministry of Research, from the Regional Council of Burgundy, from the Association Bourguignonne de Cardiologie, and from the Regional University Hospital and Faculty of Heath Sciences.

References

1. Staerk L, Sherer JA, Ko D, Benjamin EJ, Helm RH. Atrial fibrillation: Epidemiology, pathophysiology, and clinical outcomes. *Circ Res*. 2017;120:1501-1517
2. Fernandez V, Bejot Y, Zeller M, Hamblin J, Daubail B, Jacquin A, et al. Silent atrial fibrillation after ischemic stroke or transient ischemic attack: Interest of continuous ecg monitoring. *Eur Neurol*. 2014;71:313-318
3. Hachet O, Guenancia C, Stamboul K, Daubail B, Richard C, Bejot Y, et al. Frequency and predictors of stroke after acute myocardial infarction: Specific aspects of in-hospital and postdischarge events. *Stroke*. 2014;45:3514-3520
4. Gunnoo T, Hasan N, Khan MS, Slark J, Bentley P, Sharma P. Quantifying the risk of heart disease following acute ischaemic stroke: A meta-analysis of over 50,000 participants. *BMJ Open*. 2016;6:e009535
5. Ranieri M, Finsterer J, Bedini G, Parati EA, Bersano A. Takotsubo syndrome: Clinical features, pathogenesis, treatment, and relationship with cerebrovascular diseases. *Curr Neurol Neurosci Rep*. 2018;18:20
6. Oppenheimer S, Cechetto D. The insular cortex and the regulation of cardiac function. *Compr Physiol*. 2016;6:1081-1133
7. Ay H, Koroshetz WJ, Benner T, Vangel MG, Melinosky C, Arsava EM, et al. Neuroanatomic correlates of stroke-related myocardial injury. *Neurology*. 2006;66:1325-1329
8. Oppenheimer SM. Neurogenic cardiac effects of cerebrovascular disease. *Curr Opin Neurol*. 1994;7:20-24
9. Colivicchi F, Bassi A, Santini M, Caltagirone C. Cardiac autonomic derangement and arrhythmias in right-sided stroke with insular involvement. *Stroke*. 2004;35:2094-2098
10. Laowattana S, Zeger SL, Lima JA, Goodman SN, Wittstein IS, Oppenheimer SM. Left insular stroke is associated with adverse cardiac outcome. *Neurology*. 2006;66:477-483; discussion 463
11. Touze E, Varenne O, Chatellier G, Peyrard S, Rothwell PM, Mas JL. Risk of myocardial infarction and vascular death after transient ischemic attack and ischemic stroke: A systematic review and meta-analysis. *Stroke*. 2005;36:2748-2755
12. Prosser J, MacGregor L, Lees KR, Diener HC, Hacke W, Davis S, et al. Predictors of early cardiac morbidity and mortality after ischemic stroke. *Stroke*. 2007;38:2295-2302
13. Barber M, Morton JJ, Macfarlane PW, Barlow N, Roditi G, Stott DJ. Elevated troponin levels are associated with sympathoadrenal activation in acute ischaemic stroke. *Cerebrovasc Dis*. 2007;23:260-266
14. Ahn SH, Kim YH, Shin CH, Lee JS, Kim BJ, Kim YJ, et al. Cardiac vulnerability to cerebrogenic stress as a possible cause of troponin elevation in stroke. *J Am Heart Assoc*. 2016;5:e004135
15. Mochmann HC, Scheitz JF, Petzold GC, Haeusler KG, Audebert HJ, Laufs U, et al. Coronary angiographic findings in acute ischemic stroke patients with elevated cardiac troponin: The troponin elevation in acute ischemic stroke (trelas) study. *Circulation*. 2016;133:1264-1271
16. Sun L, Ai J, Wang N, Zhang R, Li J, Zhang T, et al. Cerebral ischemia elicits aberration in myocardium contractile function and intracellular calcium handling. *Cell Physiol Biochem*. 2010;26:421-430
17. Wang L, Sun L, Zhang Y, Wu H, Li C, Pan Z, et al. Ionic mechanisms underlying action potential prolongation by focal cerebral ischemia in rat ventricular myocytes. *Cell Physiol Biochem*. 2009;23:305-316
18. Min J, Farooq MU, Greenberg E, Aloka F, Bhatt A, Kassab M, et al. Cardiac dysfunction after left permanent cerebral focal ischemia: The brain and heart connection. *Stroke*. 2009;40:2560-2563

19. Ishikawa H, Tajiri N, Vasconcellos J, Kaneko Y, Mimura O, Dezawa M, et al. Ischemic stroke brain sends indirect cell death signals to the heart. *Stroke*. 2013;44:3175-3182
20. Chen J, Cui C, Yang X, Xu J, Venkat P, Zacharek A, et al. Mir-126 affects brain-heart interaction after cerebral ischemic stroke. *Transl Stroke Res*. 2017;8:374-385
21. Bieber M, Werner RA, Tanai E, Hofmann U, Higuchi T, Schuh K, et al. Stroke-induced chronic systolic dysfunction driven by sympathetic overactivity. *Ann Neurol*. 2017;82:729-743
22. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke*. 1989;20:84-91
23. Richard C, Lauzier B, Delemasure S, Talbot S, Ghibu S, Collin B, et al. Effects of angiotensin-1 converting enzyme inhibition on oxidative stress and bradykinin receptor expression during doxorubicin-induced cardiomyopathy in rats. *J Cardiovasc Pharmacol*. 2008;52:278-285
24. Li N, Guenancia C, Rigal E, Hachet O, Chollet P, Desmoulins L, et al. Short-term moderate diet restriction in adulthood can reverse oxidative, cardiovascular and metabolic alterations induced by postnatal overfeeding in mice. *Sci Rep*. 2016;6:30817
25. Hossmann KA. Experimental models for the investigation of brain ischemia. *Cardiovasc Res*. 1998;39:106-120
26. Bere Z, Obrenovitch TP, Kozak G, Bari F, Farkas E. Imaging reveals the focal area of spreading depolarizations and a variety of hemodynamic responses in a rat microembolic stroke model. *J Cereb Blood Flow Metab*. 2014;34:1695-1705
27. McBride DW, Zhang JH. Precision stroke animal models: The permanent mcao model should be the primary model, not transient mcao. [published online July 17, 2017] *Transl Stroke Res*. 2017. https://link-springer-com.gate2.inist.fr/article/10.1007%2Fs12975-017-0554-2. Accessed September 17, 2018
28. Guo S, Deng W, Xing C, Zhou Y, Ning M, Lo EH. Effects of aging, hypertension and diabetes on the mouse brain and heart vasculomes. [published online July 19, 2018] *Neurobiol Dis*. 2018 https://www.sciencedirect.com/science/article/pii/S0969996118302912?via%3Dihub; Accessed September 17, 2018
29. Kishore A, Vail A, Majid A, Dawson J, Lees KR, Tyrrell PJ, et al. Detection of atrial fibrillation after ischemic stroke or transient ischemic attack: A systematic review and meta-analysis. *Stroke*. 2014;45:520-526
30. Micheli S, Agnelli G, Caso V, Alberti A, Palmerini F, Venti M, et al. Acute myocardial infarction and heart failure in acute stroke patients: Frequency and influence on clinical outcome. *J Neurol*. 2012;259:106-110
31. Sun L, Du J, Zhang G, Zhang Y, Pan G, Wang L, et al. Aberration of l-type calcium channel in cardiac myocytes is one of the mechanisms of arrhythmia induced by cerebral ischemia. *Cell Physiol Biochem*. 2008;22:147-156
32. Cheung RT, Hachinski V. Cardiac effects of stroke. *Curr Treat Options Cardiovasc Med*. 2004;6:199-207
33. Krause T, Werner K, Fiebach JB, Villringer K, Piper SK, Haeusler KG, et al. Stroke in right dorsal anterior insular cortex is related to myocardial injury. *Ann Neurol*. 2017;81:502-511
34. Goyagi T, Horiguchi T, Nishikawa T, Tobe Y. Post-treatment with selective beta1 adrenoceptor antagonists provides neuroprotection against transient focal ischemia in rats. *Brain Res*. 2010;1343:213-217
35. Xiang Y, Zhang T, Guo J, Peng YF, Wei YS. The association of growth differentiation factor-15 gene polymorphisms with growth differentiation factor-15 serum levels and risk of ischemic stroke. *J Stroke Cerebrovasc Dis*. 2017;26:2111-2119
36. Acosta SA, Mashkouri S, Nwokoye D, Lee JY, Borlongan CV. Chronic inflammation and apoptosis propagate in ischemic cerebellum and heart of non-human primates. *Oncotarget*. 2017;8:102820-102834
37. Altintas O, Kumas M, Altintas MO. Neuroprotective effect of ischemic preconditioning via modulating the expression of adropin and oxidative markers against transient cerebral ischemia in diabetic rats. *Peptides*. 2016;79:31-38

38. Bastaki M, Huen K, Manzanillo P, Chande N, Chen C, Balmes JR, et al. Genotype-activity relationship for mn-superoxide dismutase, glutathione peroxidase 1 and catalase in humans. *Pharmacogenet Genomics*. 2006;16:279-286
39. Cadenas S. Ros and redox signaling in myocardial ischemia-reperfusion injury and cardioprotection. *Free Radic Biol Med*. 2018;117:76-89

Figure legends

Figure 1: Cardio-vascular function *in vivo* and *ex vivo*: A: Arterial systolic blood pressure after right of left cerebral embolization. B: Natural course of LV fractional shortening. *: $p<0.05$ significantly different between left stroke and sham groups and §: $p<0.05$ significantly different between right stroke and sham groups. C: LVDevP of isolated perfused rat hearts 2 h after right or left cerebral embolization during the initial minute of baseline perfusion (t0). D: LVDevP of isolated perfused rat hearts 24 h after right or left cerebral embolization during the initial minute of baseline perfusion (t0).

Figure 2: Evolution of functional parameters of isolated perfused hearts, 2 h after embolization or sham-surgery. A: Coronary flow, B: LVDP, C: LVDevP, D: -dP/dt. Hearts were perfused for 10 min at baseline and then underwent 30 min of global total normothermic ischemia followed by 1 h of reperfusion. *: $p<0.05$ significantly different between left stroke and sham groups, §: $p<0.05$ significantly different between right stroke and sham groups and £: $p<0.05$ significantly different between left stroke and right stroke groups at the same measurement time using 2-way ANOVA for repeated measures.

Figure 3: Evolution of functional parameters of isolated perfused hearts, 2 h after embolization or sham-surgery. A: Coronary flow, B: HR, C: LVDevP, D: -dP/dt. Hearts were perfused for 10 min at baseline and then underwent 20 min of global total normothermic ischemia followed by 1 h of reperfusion. *: $p<0.05$ significantly different between left stroke and sham groups and §: $p<0.05$ significantly different between right stroke and sham groups at the same measurement time using 2-way ANOVA for repeated measures.

Figure 4: Catecholamines and GDF15 plasma concentrations. A: Catecholamines levels 2 h after embolization. B Catecholamines levels 24 h after embolization. *: $p<0.05$ versus sham

groups, **: p<0.01 versus sham groups and ***: p<0.001 versus sham groups, C: GDF15 levels 2 h after embolization. D: GDF15 levels 24 h after embolization.

Figure 5: Heart eNOS (A), peNOS (B), GPX1 (C) and CAT (D) protein levels determined by western blot analysis 2 h after embolization. Data were normalized to GAPDH. n=12 per group.

Figure 6: Heart ADRB1 (A), JAK2 (B), STAT3 (C) and pSTAT3 (D) protein levels determined by western blot analysis 2 h after embolization. Data were normalized to GAPDH. n=12 per group.