Progress Report

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Title of Project:
Identifying treatment targets to improve cerebral blood flow after stroke and hypoxia.

Summary: (approximately 1,000 words):
The long-term and overall Aim of this project is to identify novel therapeutic targets to improve blood flow after ischemic brain injury; specifically in ischemic stroke. The general aim of this work is to identify the distribution and expression of selected key signalling site proteins that underlie the control and coordination of cell-cell function in specific small arteries in the brain, and how this may be altered in stroke. In addition, this project determines whether our extensive animal model data is applicable to humans.

The focus of this work is on microdomain signalling sites, where the activity of one component influences an adjacent element, as an integrated cascade of events that underlie the control of blood flow in arteries in health and disease. For example, a calcium-activated potassium channel depends on a nearby (within ~20 nm) calcium store release receptor, such as inositol-1,3,5-trisphosphate (IP_3R). The work is ongoing and will continue into 2017.

Hypothesis vs Findings:
We ***hypothesized*** that the distribution and expression of specific channels, receptors and coupling sites are altered in cerebral penetrating arteries and arterioles from humans in stroke; consistent with that seen in our animal model of ischemic stroke Such channels, receptors and coupling site proteins whose expression is being determined include; BK_{Ca}α and β1, SK_{Ca} and IK_{Ca}, TRPC3 and V4, IP_3R subtypes 1-3, and gap junction Cxs32, 37, 40, 43.

To date, we have ***found*** that;

1. The large conductance calcium-activated potassium channel (BK_{Ca})α subunit is ***absent in*** the endothelium of small penetrating cerebral arteries and arterioles of people that died of a ***non-stroke*** cause (as the control tissue), whereas they are ***present in*** the endothelium of these vessels from subjects who died of ischemic ***stroke***. These proteins are present in the smooth muscle of these vessels from control patients, with elevated expression in the vessels from stroke subjects. These data are consistent with that from our rat ischemic stroke model.
2. The calcium channels TRPC3 and V4 show differential changes in expression in the above vessel endothelium and smooth muscle of subjects that died of stroke, as well as non-stroke. These data contrast with that of our animal model of ischemic stroke.

3. Gap junction connexin (Cx)37 and 40 (but not Cxs32 and 43) expression is altered in the above vessels from stroke subjects, compared to non-stroke, control; whilst Cx32 and 43 expression remains unchanged in vessels from control compared to stroke. These data are also consistent between our animal model and human stroke and non-stroke subjects and are summarized in the Table, below.

Table. Comparative cell border connexin expression in cerebral artery of ischemic stroke and control.

Connexin	Endothelial		Smooth muscle	
	Control	Stroke	Control	Stroke
Cx32	-	-	+++	+++
Cx37	++	-	++	-
Cx40	-	+	-	++
Cx43	-	-	-	-

Expression, as; -, absent; +, low, ++, medium; +++, high.

Unanswered Questions:

Work on determining the expression and distribution of cerebral artery small (S) K_{Ca}, intermediate (I)K_{Ca}, IP_3R and Cx45 has yet to be started in the human tissues, but is anticipated to be conducted into 2017.

In addition, given that our data shows that BK_{Ca} is absent in (selected) cerebral artery endothelium in health, and present in such vessels after stroke, subject to funding, we will administer BK_{Ca} modulatory agents (low dose blockers and activators) in the cerebral circulation of the animal model, to determine their effects on recovery after stroke.

Furthermore, given that stroke changes the pressure, oxygen levels and collateral flow in affected cerebral vessels, further work will examine the effects of these variables in our animal model of stroke, and its recovery after stroke. Such animal work is essential for determining potential eventual therapy options in human stroke.

What these research outcomes mean:

This project is an evaluation of the significance and role of specific and selected signalling mechanisms in the regulation of human cerebral artery function in health and disease; and specifically in ischemic stroke. The work is a key step in identifying vascular bed, and disease specific cell signalling mechanisms, as unique potential artery-specific signalling pathways and therapeutic targets to selectively control blood flow and tissue perfusion. Our preliminary data outlined above demonstrates that there are changes in the mechanisms that control and coordinate cell-cell function in specific small arteries in the brain. As above, these data are an early step in potentially opening up new perspectives for the treatment of stroke and similar vascular disorders, with the data generated also being of significant importance for the fundamental understanding of artery function in health and disease.