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Clinical Features of Patients Who Come to Hospital at the Super Acute Phase of Stroke

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Introduction

Ischaemic stroke is common in diabetes patients and occurs due to cerebral-barrier dysfunction. Although the pathological activation of protein kinase C (PKC) is linked with the disease progression, the PKC isoform-specific downstream signalling remains obscure and is the focus of this study which explored the clinical relevance of PKC-α inhibitor Ro-32-0432 under hyperglycaemia (HG).

Methods and Findings: Total PKC and RhoA activities were studied in human brain microvascular endothelial cells (HBMEC) exposed to normoglycaemia or HG. The integrity and function of an in vitro model of human BBB composed of HBMEC and human astrocytes were measured by transendothelial electrical resistance (TEER) and paracellular flux of Evans blue-labelled albumin (EBA), respectively. Exposure of HBMEC to HG for 72 hours led to significant increases in activities of total PKC and RhoA as well as in mono- and di-phosphorylation of MLC2 which concurred with substantial decreases in TEER and marked elevations in barrier permeability. Enhanced cellular contractility triggered by actin stress fibre formation appeared to further potentiate HG-mediated barrier dysfunction. Pre-treatment of HBMEC with Ro-32-0432 or transient PKC-α protein knockdown led to effective preservation of BBB integrity and function in hyperglycaemic settings by suppressing RhoA activity and subsequently normalising the MLC2 phosphorylation on Ser19 and Thr18- Ser19 residues. These observations were further supported by disappearance of stress fibres and subsequent restoration of the cortical actin staining.

Conclusions: Neutralisation of PKC-α activity may be of considerable therapeutic value in clinical settings accompanied by diabetes or stress HG.

The vascular endothelium lines the entire inner surface of all blood vessels and forms specific barriers in different organs to regulate vascular permeability. The blood-brain barrier (BBB) represents one such barrier and prevents the leakage of bloodborne substances into brain parenchyma. Disruption of the BBB characterised by the formation of brain oedema is a common occurrence after ischaemic strokes and constitutes the main cause of mortality within the first week after a cerebrovascular attack. As the brain oedema is more prevalent in stroke patients with diabetes mellitus, it is thought that hyperglycaemia may be intricately involved in the pathogenesis of this defect .

The BBB consists of brain microvascular endothelial cells (BMEC), capillary basement membrane and astrocyte end-feet supporting the basement membrane. The permeability of the BBB is tightly regulated by tight junction proteins, notably occludin and claudin-5, that exist between the adjacent BMEC. Hence, pathologies that affect BMEC phenotype and/or tight junction protein expression and localisation would have an adverse effect on the integrity and function of the BBB. Although several biochemical pathways including non-enzymatic glycation of proteins and lipids with the irreversible deposition of advanced glycation end products, the activation of polyol pathway or stimulation of protein kinase C (PKC) pathway have been implicated in diabetes-mediated peripheral vasculopathies, the mechanisms involved in hyperglycaemia-evoked cerebrovascular impairments remain largely unknown .

In addition to regulation of PKC activity, the suppression of RhoA may also be an efficacious therapeutic option in protecting cerebral barrier integrity given the fact that binding of active RhoA to its downstream effector Rho-kinase leads to the disruption of intercellular junctions by sequential induction of myosin-regulatory light chain-2 phosphorylation (pMLC2) and actin stress fibre formation .

In light of the above, this study investigated the specific role of PKC-α isoform in hyperglycaemia-mediated cerebral damage using an *in vitro* model of human BBB. In addition, comprehensive analyses of the relationship between PKC-α isoform and RhoA/ pMLC2 pathway were performed to unravel new therapeutic targets that can be utilised to prevent or minimise cerebral barrier damage in hyperglycaemic settings.

Keywords: Endothelial cell; Hyperglycaemia; Protein kinase C; Ro-32-0432; RhoA; cytoskeletal remodeling