Expression of the Endocannabinoid Receptor 1 in Human Stroke: An Autoptic Study

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Objective: Stroke is one of the leading causes of disability and death in the world. The endocannabinoid (eCB) system is upregulated in several neurological diseases including stroke. A previous animal study demonstrated an increased expression of the endocannabinoid receptor 1 (CB1R) in the penumbra area surrounding the ischemic core, suggesting a crucial role in inflammation/reperfusion after stroke. Regarding the localization of CB1/CB2 receptors, animal studies showed that cortical neurons, activated microglia, and astroglia are involved. Our aim was to evaluate the cerebral expression of CB1R in the ischemic brain areas of 9 patients who died due to acute cerebral infarction in the middle cerebral artery territory. *Methods:* The cerebral autoptic tissue was collected within 48 hours since death. Ischemic and contralateral normal-appearing areas were identified. After tissue preprocessing, 4-μm-thick cerebral sections were incubated with the primary CB1R antibodies (Cayman Chemical Company, Ann Arbor, MI). Thereafter, all cerebral sections were hematoxylin treated. In each section, the total cell number and CB1R-positive cells were counted and the CB1R-positive cell count ratio was calculated. For statistical analysis, Student's t-test was used. *Results:* In normal tissue, CB1R-positive neurons were the majority; a few non-neuronal cells expressed CB1R. In the ischemic areas, a few neurons were detectable. A significant increase in total CB1R staining was found in the ischemic regions compared to contralateral areas. *Conclusions:* We found an increase in CB1R expression in the ischemic region (neuronal and non-neuronal cell staining), suggesting the inflammatory reaction to the ischemic insult. Whether such response might mediate neuroprotective actions or excitotoxicity-related detrimental effects is still unclear. **Key Words:** Ischemic stroke—cannabinoid receptors—inflammation—autoptic study—ischemic core—penumbra.

Introduction

Stroke is one of the leading causes of disability and death all over the world. Ischemic stroke alone represents 85% of acute strokes. Ischemic stroke is due to a critical regional reduction of cerebral blood flow induced by a vascular occlusion, either large-vessel thrombosis or embolization of a clot. Subsequent ischemia triggers a complex series of molecular cascades involving free radical formation, excitatory damage, mithocondrial failure, intracellular calcium increase, lipid peroxidation, spreading depression, inducible nitric oxide synthase activation, inflammation, blood–brain barrier (BBB) disruption, and vasogenic edema, leading to necrosis and apoptosis of the affected brain tissue.[1,2]

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It is well known that cannabinoid receptors and endocannabinoid (eCB) levels are elevated by stroke, with potential neuroprotective effects. Moreover, some eCBs have been shown to play a role in the regulation of the BBB permeability also in other conditions than ischemia; the endocannabinoid system (ECS) was actually found to play an important modulatory role in normal BBB physiology and also to afford protection to the BBB during ischemic stroke.[3]

eCBs are lipid mediators synthesized on demand that act on specific (endocannabinoid receptor 1 [CB1R] and endocannabinoid receptor 2 [CB2R]), nonspecific, and other (transient receptor potential vanilloid receptor) receptors. Presynaptically, activation of CB1R inhibits neurotransmitters' release (glutamate and gamma-aminobutyric acid).[4] Postsynaptycally, CB1R and CB2R activation mostly reduces the intracellular levels of Ca^{2+} and modulates the mitogen-activated protein kinase pathway.[5] CB1Rs are highly expressed in the central nervous system (i.e., basal ganglia, hippocampus, cerebellum, and neocortex), but also in peripheral tissues; CB2Rs are more expressed by immune cells, including brain resident microglial cells and hematopoietic cells.[6-8]

The hypothesis that the endocannabinoid signaling system (ECS) is involved in ischemic injury is confirmed by several observations: first of all, the eCBs and related lipids accumulate in ischemic tissues,[9,10] supporting the hypothesis that the ECS is activated during ischemia. Moreover, the role of ECS in metabolic homeostasis[11] and responsivity of the brain to stress[12] has been detected in several studies. Finally, it is known that activation of the CB1 cannabinoid receptor leads to a reduced probability of opening of voltage-operated calcium channels[13] with the consequent reduction in intraneuronal calcium contents. In addition, CB1 receptor activation results in inhibition of glutamate release in response to depolarization.[14] Thereby the ECS could exert a neuroprotective role in stroke (ECS is involved in neuroprotection, leading to a low concentration of intracellular calcium and glutamate). The CB1 cannabinoid receptor is present in the cerebral vasculature and its activation produces vasodilation.[15,16]

Regarding the CB2 cannabinoid receptors, is well known that CB2Rs are expressed by immune cells, including brain resident microglial cells, and their activation results in a decrease in the release of proinflammatory mediators.[17] Despite human and animal studies suggesting the presence of CB2Rs in the cerebellum and the brain stem, their expression in the cortex remains to be conclusively demonstrated.[18]

In the past several years, several animal studies indicated a possible protective role of the ECS in different neurological conditions and in stroke.[19,20] Particularly, CB1R activation reduces glutamate-induced excitotoxicity by inducing hyperpolarization of the neuronal membrane[21] and by directly inhibiting voltage-gated calcium channels,[22] thus determining a decrease in intracellular calcium concentration and glutamate.[23,24]

A previous animal study demonstrated an increased expression of CB1 receptor in the penumbra area surrounding the central ischemic core after middle cerebral artery (MCA) occlusion,[25-27] suggesting an early involvement of the ECS during the acute phase of stroke, lasting from 2 to 72 hours.

The localization of cannabinoid CB1 and CB2 receptors in rat brains before and after focal cerebral ischemia (due to transient occlusion of the MCA) has been investigated by Schmidt et al[18] in a murine stroke model; both receptor subtypes were identified in cortical neurons. These results were confirmed in a rat phototrombotic stroke model, where an increase of CB1R-specific positron emission tomography (PET) activity was found in the peri-infarcted areas during the acute and subacute phases, while no differences in CB2R staining were reported. Data showed a time-dependent and regionally strong increase in CB1 as a consequence of photothrombotic stroke, but not of CB2, concluding that any pharmacological interventions should primarily aim at CB1 signaling.[28]

Elsewhere, it was demonstrated that eCBs via the CB1 receptor exert a regulatory role on neural progenitor cell proliferation and differentiation, showing, together with the ability of cannabinergic drugs to modulate neurogenesis after brain damage, a possible function in response to excitotoxicity or ischemia.[29]

The aim of our study was to evaluate in human brain tissue the changes of cortical expression of CB1R in the ischemic brain areas, compared to the contralateral healthy brain tissue, during the subacute phase of a first acute cerebral infarction in the MCA territory.

Methods

Subjects

Postmortem human brain samples were obtained from 9 Caucasian patients (2 males and 7 females; mean age ± standard deviation: 77 ± 5.2 years) who died within 14 days after a first ischemic stroke involving the MCA territory within 6 hours since symptom onset; the patients were hospitalized to our stroke unit between 2010 and 2011.

Stroke patients were chosen among a group of 20 subjects who had died as a consequence of the acute cerebral infarction; the subjects were selected on the basis of the following exclusion criteria: history of any neuropsychiatric disorder, previous alcohol and/or substance abuse, systemic inflammatory diseases or tumors, and use of any antidiabetic or lipid-lowering drugs in the last 6 months preceding stroke onset. All the patients had at least 1 risk factor for stroke, mainly hypertension, diabetes, atrial fibrillation, and hypercholesterolemia, even though they did not assume specific drugs at home.