ALZHEIMER-LIKE STRUCTURAL CHANGES IN THE RAT BRAIN INDUCED BY INTRACEREBROVENTRICULAR ADMINISTRATION OF STREPTOZOTOCIN

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Alzheimer disease (AD) is an age-related heterogeneous neurodegenerative disorder. There are two types of AD; early-onset familial AD caused by mutation of genes leading to an increased formation of amyloid precursor protein (APP) derive amyloid beta (Aβ) peptid, and the late-onset sporadic AD, the etiopathogenesis of which is still unclear ¹. Clinically, it is characterized by loss of memory and progressive dementia. Histopathologically, two main changes are found in the brain of patients with AD: neurofibrillary tangles (NFT) and amyloid plaques ¹. NFT consist of intracellular deposits of abnormally hyperphosphorylated tau protein ². Amyloid plaques are generated by an extracellular deposition of fibrils of Aβ, which are derived from the proteolytic processing of APP ³. APP is a transmembrane protein that, following the enzymatic processing by secretases, produces various fragments. Amyloid deposits consist of aggregates of Aβ containing 40 or 42 residues, with Aβ1-40 being the most abundant derivative, and Aβ1-42 being less abundant, but pathogenically important ³. Currently, it is widely accepted that Aβ metabolism misbalance is crucial for AD progression ³. However, because of the specific nature of AD pathology, neurochemical and histopathological analysis of amyloid plaques in the brain is done post-mortally, generally in advanced stage of disease, with difficulties in investigating the time-course of changes. Extensive research has been focused on finding the experimental model that would be a truly representative of the sporadic type of AD, and not related to gene manipulation. Sporadic AD has now been recognized as an insulin resistant brain state, and streptozotocin-intracerebroventricularly (STZ-icv) treated rats seem to be one of its most appropriate experimental models, because STZ is a drug selectively toxic for insulin producing/secreting cells ⁴. While peripheral STZ administration in high doses induces diabetes mellitus in rats ⁵, central administration of very low STZ doses does not produce diabetes mellitus, but produces neurochemical and brain glucose metabolism changes, as well as long-term and progressive deficit in learning, memory and cognitive behavior, that resemble those found in the brain of patients with AD ⁴. Recently we have reported altered expression of enzymes downstream the insulin receptor (IR) signaling pathway at the level of protein kinase B and glycogen synthase kinase-3 (GSK-3α/β) in the hippocampus of STZ-icv treated rats, three months after STZ-icv treatment ⁶, which could have important implications on substrates further on in the signaling cascade in relation to the fact that GSK-3β isoform is involved in the phosphorylation of tau protein and GSK-3α isoform is involved in aggregation of Aβ ⁷. The scope of our experiments was to investigate the possible histopathological changes at the level of Aβ in the rat brain three months following the STZ-icv treatment.

Three-month-old male Wistar rats weighing 280-330 g (Department of Pharmacology, School of Medicine, University of Zagreb) were used throughout the study, and kept on standardized food pellets and water ad libitum. Rats were randomly divided in 2 groups (6 per group) and given
general anaesthesia (chloralhydrate 300 mg/kg, ip), followed by drug injection icv bilaterally into the lateral ventricle (2 μL/ventricle), according to the procedure described by Noble et al. 8. STZ (1 mg/kg, dissolved in 0.05M citrate buffer pH 4.5) was applied to one group, and an equal volume of vehicle icv to another group of animals. Cognitive functions tested by Morris Water Maze Swimming Test 9 confirmed deficits in learning and memory. Animals were sacrificed three months after the drug treatment. Drug treatments and behavioural tests were carried out in Croatia under the guidelines of The Principles of Laboratory Animal Care (NIH Publication No. 86-23, revised in 1985), according to the Croatian Act on Animal Welfare (NN 19/1999), and were approved by The Ethics Committee of the Zagreb University School of Medicine (No. 04-7672005-54). Beta-amyloid fibrils in the brain were visualized by a modified alkaline Congo Red staining 10. Briefly, pre-fixed frozen tissue sections (6 μm) were air dried for one hour. The sections were then incubated for 15 min in Mayer’s Hematoxylin Solution and rinsed in tap water to display the nuclei. Subsequently, the tissue slices were incubated for 20 min in 80 % (v/v) ethanol, 3 % (m/v) NaCl, 1 % NaOH, to be then transferred to the Alkaline Congo Red Solution containing 80 % (v/v) ethanol, 3 % (m/v) NaCl, 1% NaOH, 0.5 % (m/v) Congo Red. For stain development, the slices were twice briefly rinsed in absolute ethanol and incubated for 5 min in xylene. Green autofluorescence was visualized in cross-polarized light.

In light microscopy of brain tissue sections of STZ-icv treated rats, some diffuse congophilic deposits were found in the brain capillaries (Fig. 1A). As Congo Red is known to specifically bind to Aβ fibrils 11, and has been widely used in histological staining procedures for the evaluation of β-amyloid aggregates in human 12 and murine 13, congophilic deposits found in STZ-icv treated rats could be considered as Aβ peptide-like aggregates. Congophilic deposits were afterwards checked with cross-polarized light, where they showed characteristic green autofluorescence (Fig. 1B). Contrary to that, no autofluorescence signal was recorded in brain tissue sections of control rats (Fig. 1C and D).

STZ-icv treated rats have been used for investigations of the brain glucose/energy metabolism alterations associated with cognitive deficits, based on which they have been proposed as a probable experimental model of sporadic-type of AD 4. However, convincing evidence regarding the pathognomonic hallmarks of AD, i.e. NFT and amyloid plaques that could clearly define connection of this experimental model to the structural changes found in the brain of people with AD, has still been missing. Therefore, diffuse congophilic deposits in the brain capillaries of STZ-icv rats that demonstrate green autofluorescence in cross-polarized light are important evidence suggesting histopathological resembling between this experimental animal model and human sporadic type of AD, at the level of Aβ. Namely, this finding is in line with the data of cerebral amyloid angiopathy, defined by Aβ depositions in cerebral vessels, associated with AD 14. Aβ-like aggregates could not be found elsewhere in the brain tissue except in the capillary wall three months following the STZ-icv treatment, which may indicate an early stage of Alzheimer-like changes in these animals. Our results not only support the hypothesis that STZ-icv rats represent a valuable experimental model of sporadic AD, but also suggest that they could be a useful animal model for monitoring development of changes at various stages of disease, and correlate them with cognitive deficits observed as early as one month following the STZ-icv treatment, and, in addition with alterations of brain insulin receptor signaling cascade demonstrated in STZ-icv treated rats 6.
Fig. 1. β-Amyloid peptide deposits in meningeal capillaries of STZ-icv treated rats 3 months after drug treatment, visualized by Congo Red staining (A) and green autofluorescence in cross-polarized light (B). Control animals (C and D). Magnification: 20x.


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