Alzheimer lesions after ischemia-reperfusion brain injury

Ryszard Pluta

Department of Neurodegenerative Disorders, Medical Research Centre, Polish Academy of Sciences, Warszawa and Department of Biological and Medical Sciences, Pedagogical University, Częstochowa, Poland

For now the best-established and accepted theory in Alzheimer’s disease (AD) etiology by most scientists is the “amyloid theory”, as the main molecular factor of neurodegeneration in AD. We critically review these observations and highlight inconsistencies between the predictions of the “amyloid hypothesis” and the published data. The research of neurobiology of AD, now more than ever, needs an infusion of new concepts. Handful researchers now recognize brain ischemia as a prominent feature in AD and a potential target for therapy aimed at treatment and prevention of disease. The “ischemia-reperfusion theory” was primarily aimed at stimulating study and redirecting the focus of investigations towards ischemic cellular mechanisms of AD. To accommodate the recent progress of study in AD there is a need to synthesize all the divergent pieces of data into a coherent story. This review provides a synopsis of current information about ischemic cellular and molecular mediators involved in Alzheimer’s neuropathology as well as interactions between these mediators that influence pathology. In this paper, current knowledge on the close relation between vascular disease factors and Alzheimer’s type dementia will be reviewed. We will summarize the data with a special focus on Alzheimer lesions in experimental brain ischemia. Taken all together, evidence presented in this review suggests a scheme for Alzheimer’s pathogenesis with ischemia playing a crucial role in influencing and linking β-amyloid deposition to neuronal damage and clinical disease.

key words: Alzheimer’s disease, brain ischemia, reperfusion injury, β-amyloid peptide, apolipoprotein, presenilin, tau, neurodegeneration, inflammation, vaccination

INTRODUCTION

Perhaps the most important questions posed by the neurobiology of dementia concern the pathogenic mechanisms in Alzheimer’s disease (AD). Alzheimer’s disease is a progressive dementia affecting a large proportion of the ageing society. Currently, this disease affects 4 million Americans and this number will grow to 14 million by 2050 unless effective treatments are found. Worldwide, these numbers are far greater. Alzheimer’s disease currently costs the United States an estimated 100-billion dollar a year from medical care charges and informal care. It is estimated that the average cost of care is between 40 000 to 60 000 dollars per patient per year with nearly 500 000 newly diagnosed patients every year. Current efforts to develop treatments to delay the
symptoms of AD are not adequate [3, 4, 33–36, 38] to overcome the demographic forces that are increasing the total number of affected individuals.

The clear majority of AD cases is sporadic and may develop from a myriad of suggested causes [11, 12, 29, 37]. In this situation the mechanisms underlying the development of AD are not well understood and are the subject of much debate and innumerable publications. A lot of attention has been focused on the neuropathological changes in AD, including the formation of amyloid plaques and neurofibrillary tangles and widespread neuronal cell death especially in hippocampus [11, 12]. The major component of the amyloid plaques, which accumulate extracellularly in the AD brain, is β-amyloid peptide (βA). “Amyloid theory” of AD is based on the hypothesis that βA toxicity [10] and deposition [8] are central to the etiology of AD [11]. Numerous experimental studies suggest that βA is a major plaque protein that develops toxic and inflammatory βA fibrils in the brain [8, 10–12]. Some studies indicate that βA1-42 bidirectionally crosses the blood-brain barrier (BBB) [27, 31, 39] and once within the brain it may contribute to plaque development [27]. Other investigations have shown that minute amounts of βA, especially of βA1–42 may seed toxic fibril formation [10]. Together, these effects within vessel walls and brain parenchyma may enhance the pathology of AD although brain amyloidosis of transgenic mice overexpressing amyloid precursor protein (APP) has only some of the neuropathology noted in AD [8]. Amyloid plaques are observed, but cell loss is minimal and neurofibrillary tangles are absent [8]. Beta-amyloid peptide deposits formed by overexpression of APP in transgenic mice do not cause sufficient neuronal cell death although the amyloid deposits are associated with gliosis and neuritic dystrophy. This suggests that additional factors are necessary to promote the progression of the disease.

In the last ten years, new research focused on the critical role of the cerebrovascular diseases in the pathogenesis and evolution of AD [2, 9, 13, 14, 16–18, 25, 28, 32, 43, 45, 49]. Growing experimental evidence implicates a dysfunctional cerebrovasculature to amyloid formation, loss of neuronal function and cognitive decline. In animal models, disturbed neuronal function has been shown to lead to neuronal damage and death, suggesting that ischemic abnormalities are very relevant to the evolution of AD. Interestingly, Alzheimer, in his own report presenting changes in the brain of the first case, had described that besides storage of peculiar material in the brain endothelial proliferation as well as occasional neovascularisation were visible [1]. Endothelial proliferation and angiogenesis and moderate arteriosclerosis in the brain arteries of the first patient [1] provide evidence that cerebrovascular lesions were also evident in the original case of Alzheimer, which now defines AD. The influence of the cerebral microvasculature on neuronal function in AD introduces novel treatment approaches for Alzheimer’s type dementia.

The objectives are to discuss how the ischemic pathological mechanisms in AD cause specific changes and how these changes yield the cognitive decline. In this paper current experimental knowledge on the relation between cerebrovascular disease/ischemia factors and Alzheimer’s type lesions will be reviewed.

**ALZHEIMER’S PROTEINS IN ISCHEMIA-REPERFUSION INJURY**

**Amyloid precursor protein**

Animals after brain ischemia-reperfusion injury showed increased brain immunoreactivity to the N- and C-terminal of APP as well as to the βA [2, 13, 14, 16, 18–21, 25, 28, 30, 32, 37, 41, 42, 49]. The immunoreactivity was observed intracellularly [2, 13, 14, 18–21, 49] within neuronal and glial cells but also extracellularly in the perivascular and nonperivascular areas [16, 25, 28, 32, 37]. Post-ischemic brains showed widespread and multifocal diffuse APP/βA plaques predominantly in the hippocampus, cerebral and entorhinal cortex, and corpus callosum or around the lateral ventricles [25, 37]. Multiple, abundant, extracellular APP/βA deposits embraced or adjoined the blood vessels, mainly capillaries spreading multifocally outward into the parenchyma. Perivascular deposits formed irregular, often asymmetric, well-delineated zones, which frequently encircled vessels, forming round, perivascular cuffs or halo. Diffuse, broad, but faintly positive perivascular areas were also seen. Endothelial, pericyte and ependymal and glial cells [2, 20, 21, 25, 28, 45] were labeled too. Staining was seen mainly in undamaged cells. Animals with short survival manifested the strongest labeling to βA and CAPP and weaker labeling to NAPP [25]. Rats with long survival revealed increased brain staining only to the CAPP as well as to the βA region [37, 49].

**Apolipoproteins**

The overexpression of apolipoproteins (Apo) following ischemia was observed not only intracellularly [13, 14, 17], but also extracellularly [16, 17, 30, 37, 38] in the perivascular and nonperivascular areas [17]. Extracellular ApoE, J and A1 positive regions were well delineated, irregular, and embraced or adjoined mainly the capillaries [17]. Diffuse, broad, but faintly apolipoproteins positive perivascular or nonperivascular areas were
also seen. Strong apolipoproteins staining was noted also in irregular, spider-like, acellular necrotic foci [17]. Staining was seen mainly in damaged cells, predominantly in neurons.

**Presenilins**

Expression of presenilins after ischemia-reperfusion brain injury was more marked in a trace of the pyramidal cells of the ischemic focus [40]. Presenilin genes are induced in the cortex and hippocampus after ischemic brain injury [22, 46], suggesting that these Alzheimer’s disease related genes might well be components of a brain injury response.

**Tau**

Neurons and oligodendrocytes containing tau protein were seen in the thalamus, hippocampus and cortex following ischemia-reperfusion brain injury [6, 7, 9, 15, 45].

**INFLAMMATION IN ISCHEMIA-REPERFUSION INJURY**

The brain is in many ways an immunologically privileged organ, but following ischemia many inflammatory molecules, such as cytokines are noted predominantly in affected areas of the brain [5, 44, 47]. These molecules can have either beneficial and/or detrimental effects. Also, as mentioned previously, the βA identified in ischemic brain itself may potentially lead to central nervous system inflammation [12]. In addition to numerous cytokines and their receptors, including interleukin 1-β and tumor necrosis factor are upregulated in ischemic brain [5, 44, 47]. As for other inflammatory molecules, several acute phase compounds, such as Apo E [16, 17] are associated with ischemia-reperfusion brain injury. Apolipoprotein E enhances microglial expression of various inflammatory compounds. Not only is there a complex interaction between these molecules, but also many of them have been presented to act specifically with APP and its fragments [12, 29]. These interactions are reciprocal, which may result in a vicious cycle developing post-ischemic encephalopathy with dementia. Studies of the role of these inflammatory compounds in AD are possible on an animal ischemic model with almost all the features of the disease.

**ALZHEIMER’S NEUROPATHOLOGY IN ISCHEMIA-REPERFUSION INJURY**

In animals with short-term survival after brain ischemia-reperfusion injury, vessel wall pathology includes: appearance of a variety of vasospastic events within the cerebral blood vessel wall, increased numbers of endothelial microvillar formations, cellular invaginations, microthrombus formation, and focal increase in vessel permeability for cellular and non-cellular blood components [23, 24, 26, 48]. Animals with long-term survival following brain ischemia showed delayed/chronic dysfunction and random BBB changes [43]. Permeability alterations were spotty and dispersedly in cortex, hippocampus, thalamus, basal ganglia, cerebellum and white matter. Peroxidase extravasations involved microcirculation, venules, veins and arterioles. Plenty of APP deposits were associated with the vessels. Perivascular deposits of amyloid took the same form as extravasated peroxidase [43]. Additionally, investigations revealed numerous platelet aggregates of varying sizes within both the arterial and the venous intraparenchymal vessels [26]. Aggregates of platelets, such as BBB changes and APP deposits, were focal, random and dispersed. Some platelets were found outside the brain vessels in the perivascular space [26, 43]. Blood-brain barrier changes and platelet aggregation correlated well with APP deposits [43]. Blood-brain barrier permeability for cellular and non-cellular blood components continues well beyond the acute stage. The profile of microvascular pathology that is observed in an experimental rat model of global brain ischemia shares a commonality with neurodegenerative processes in AD. Chronic BBB dysfunction, platelet-vessel wall interactions, and platelets in the perivascular space with APP accumulation may be involved in the gradual maturation of injurious process leading, over a lifetime, to cumulative, repeated and silent cerebral reinfarctions, which cause a slowly progressing post-ischemic encephalopathy with dementia. These consequences, as carried to the extreme in ischemic brain, can initiate a chain reaction that leads to further neuronal insults, further βA accumulation, and to subsequent Alzheimer-type neuropathology formation.

Investigations on neuronal loss after ischemia are largely centered on the hippocampus because it is the part of the brain that bears the brunt of AD pathology. Few days following ischemic brain injury, numerous small areas of pyramidal neurons necrosis were noted in the CA1 sector of hippocampus. Complete disappearance of the pyramidal neurons in the CA1 region was seen 7 or 14 days later [37, 41]. The disappearance of the pyramidal neurons was localized largely in the hippocampus and third cortical layer [37]. In addition, some surviving pyramidal cells were seen scattered in the necrotic CA1 sector. About one-third of the investigated ischemic brains did not show disappearance of CA1 area in early stages [37]. They developed loss of neurons of CA1 region in long-term survival periods of more than
1 month. The present data demonstrate remarkable preservation of tissue integrity especially in CA1 sector as well as distinct pathology in brain areas considered to be resistant to ischemic injury. The CA2, CA3 and CA4 areas of the hippocampus and dentate gyrus showed early postischemic pathology of neurons 1, 6, 9 and 12 months after brain ischemia [37]. In long-term survival time points, degenerative changes in neuronal cells in brain took the form of “burn faintly phenomenon” [41]. More recently, it has become recognized that neuropathological processes continue well beyond the acute stages.

Studies indicate that transient brain ischemia-reperfusion injury in animals is followed by widespread neuronal damage, involving all brain structures, belonging or not belonging to selectively vulnerable areas of the brain. In general, the intensity of structural changes revealed marked individual variability. The postischemic structural brain alterations represent a gradually progressing process extending over a long period after the ischemic episode. Transient brain ischemia in the rat produces a stereotyped pattern of selective neuronal damage, involving all brain structures, belonging or not belonging to selectively vulnerable areas of the brain. In general, the intensity of structural changes revealed marked individual variability. The postischemic structural brain alterations represent a gradually progressing process extending over a long period after the ischemic episode. Transient brain ischemia in the rat produces a stereotyped pattern of selective neuronal degeneration, which indicates early AD pathology.

In some animals with long-term survival following brain ischemia-reperfusion episode, widened sulci and flattened atrophic gyri are observed. An irregular contour of the hemisphere may also be seen. Neuropathological gross examination performed 9–12 months after ischemic injury of brain revealed hydrocephalic features of brain and dilatation of the subarachnoid space around and between the brain hemispheres. Almost complete atrophy of the dorsal hippocampus with disappearance of the CA1 area and atrophy of the striatum were observed [28, 37, 42]. Brain cortex in most areas was narrow showing increased neuronal density. White matter revealed advanced spongiosis leading to profound cavitations. Some animals following brain ischemia-reperfusion developed brain atrophy, which is indicative of an active, slowly progressing pathological process.

**TREATMENT OF AMYLOIDOSIS**

In summary, if βA deposition is now critical to the dementia in AD, amyloid mechanisms also appear to be implicated in the neuropathology of ischemic dementia as an additional factor. Given the potential complexity of these interactions, therapies against βA [30, 38] may have an important role in vascular dementia including post-ischemic syndromes and mixed dementias along with AD. For the first time, we have presented data on a time dependent complete disappearance of diffuse amyloid plaques following immunization under experimental conditions [33–35]. Treatment by vaccination actively stops formation of amyloid deposits and prevents their maturation. Additional observations suggest that a reverse transport of βA across BBB is possible for removal of βA from the brain parenchyma [39]. These findings indicate that in normal conditions βA is rapidly cleared by endothelial cells from the brain parenchyma, suggesting that they are involved in βA deposition in the brain in AD.

**CONCLUSIONS**

The question is, do cerebrovascular changes precede or follow the clinical onset of AD? The answer is that ischemia-reperfusion brain injury creates a vicious cycle of neurodegeneration in AD. These data indicate that there are long-lasting alterations of Alzheimer proteins after brain ischemia-reperfusion injury. Thus, these derivatives are likely important intermediates in the pathway leading to fibrillary amyloid deposition in AD. But we have shown that soluble βA, even without fibrillar βA, can devastate neurons following brain ischemia-reperfusion. The presented data suggest that the level of fragments or the full-length APP in cerebral parenchyma could be regulated by BBB sequestration and transport of their respective circulating precursor. These results when taken together clearly suggest that βA deposition follows neuronal death. All these results suggest that β-amyloidogenesis may involve a process that is also activated in ischemia-reperfusion brain injury. Moreover, ischemia-like conditions may contribute to pathogenic βA production and accumulation. Alternatively, the presence of new microinfarcts in rat model with long-term survival might be explained by development of variability in cerebral blood flow with time. Acute and chronic ischemic brain injury may model some of the mechanisms, which are associated with the development of AD pathology. Finally, we can conclude that ischemia importantly contributes to the onset and progression of AD neurodegeneration over years.

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**REFERENCES**


