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INVOLVEMENT OF COMPLEX CARBOHYDRATE CHEMISTRY IN ALZHEIMER DISEASE

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REVIEW

ABSTRACT. THE CARDINAL FEATURE OF Alzheimer disease is the extracellular deposition of proteinaceous amyloid-β fibrils as senile plaques. Amyloid-β plays an essential role in disease diagnosis and is also thought by many to be a key mediator of disease pathogenesis. As such, there are tremendous efforts underway to understand mechanisms of amyloid deposition. In this context, it is notable that the actual term amyloid, represents a historical misnomer (being derived from amylose, i.e., starch) and since this realization, the contribution of carbohydrates in disease pathogenesis has largely been ignored. However, recently, two emerging lines of evidence indicate not only that the interaction of carbohydrates with amyloid is a key event in disease pathogenesis but also that therapeutic efforts targeted towards such pathways may prove therapeutically efficacious. First, just over a decade ago, we and others discovered that oxidative glycation, similar if not identical to that found in diabetes, was an early and chronic contributor to the disease. Second, we very recently found evidence for the presence of chitin-like polysaccharides in association with amyloid deposits in the diseased brain. Both carbohydrate-associated changes likely contribute to the physiochemical properties of amyloid (and other disease-related proteins such as tau) and, as such, to the insolubility and protease-resistance of amyloid. In fact, taken together, the findings indicate an emerging and important role for carbohydrates in the pathogenesis of Alzheimer disease.

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ALZHEIMER DISEASE

Initially described in 1907 by Alois Alzheimer, this condition, which now bears his name, describes a fatal neurodegenerative disorder that starts with mild memory impairment and poor judgment but progresses to apraxia, aphasia, and agnosia. The latter stages are often characterized by complete debilitation, requiring constant intensive supervision. Following initial diagnosis, the course of the disease varies from a few years to over 20 years, with an average of 4 to 8 years. Alzheimer's disease (AD) is by far the most common cause of senile dementia.

Originally, AD was viewed as two distinct clinical conditions, depending on the age of onset. Alzheimer's disease was a term reserved for presenile (<65 years) dementia and senile dementia of the Alzheimer-type was used to describe the condition in elderly (>65 years) patients. Although these classifications are still used in reference to age of onset, AD fails to demonstrate either a bimodal age of onset or phenotypic differences and is therefore recognized as a single entity with a prevalence that increases sharply with age. Rare cases of AD have been reported from the second and third decades of life; however, the vast majority of patients are over the age of 60 such that, within the United States, 10% of the population older than 65 years and as many as 47% of those older than 85 years are afflicted.

The distinctive brain lesions, senile plaques and neurofibrillary tangles, used by Alois Alzheimer together with the clinical deficits to describe the disease, are still used today as the defining features for diagnosis. In addition to these striking changes, there is variable cerebral cortical atrophy, particularly of the temporal and frontal lobes, and associated ventricular dilation, both of which are consequences of the neuron loss and astrocyte proliferation in affected regions.

Both neurofibrillary tangles and senile plaques are found in normal aged persons, but it is their quantitative increase that defines the pathologic diagnosis of AD. Neurofibrillary tangles consist of abnormal 20-nm helical filaments with an 80-nm half-periodicity, termed paired helical filaments, and 12- to 15-nm straight filaments. Paired helical and straight filaments occur not only in neuronal perikaryon as neurofibrillary tangles, but also within dendrites and axons as neuropil threads, and within synaptic terminals of the large dystrophic neurites surrounding the amyloid-β deposits of senile plaques. The amyloid-β of senile plaques consists of 7- to 10-nm helical filaments that share with paired helical filaments the ability to bind the dye Congo red and appear birefringent green when viewed under cross-polarized light, a property of β-pleated molecular sheets. Amyloid-β deposits in senile plaques are found almost exclusively in cortical and subcortical gray matter, where they can take the form of either closely opposed filaments termed cores or loose deposits termed diffuse. Amyloid-β is also found in meningeal and gray-matter blood vessels, termed congophilic angiopathy, although the pathobiologic significance of vascular amyloid-β is unclear.

CARBOHYDRATES AND ALZHEIMER DISEASE

Studies related to the role of carbohydrates in the pathophysiology of amyloidosis date back to Virchow [1] and the introduction of the term "corpora amylacea" (amyloid bodies) for microscopic lesions within the brain and spinal cord that demonstrated an affinity for iodine. Only a few years later Friedreich and Kekule [2] used a similar term "amyloid" to describe extracellular accumulations in peripheral organs. It is now apparent that Virchow was describing CNS corpora amylacea, being intraastrocytic accumulations of carbohydrate material and was correct in his interpretation that the material reacted chemically like starch. On the other hand, the "amyloid" as assessed by Friedreich and Kekule corresponds to amyloid as it is known currently, being a collection of extracellular, insoluble fibrils considered largely proteinaceous in composition, with positive staining for thioflavin, and "apple green" birefringence on plane polarized light following Congo red staining [3]. Noteworthy examples of proteins capable for forming amyloid include amyloid-β, prion protein, transthyretin, se-

rum amyloid A, immunoglobulin light chain, and gelsolin.

Friedreich and Kekule appropriately disagreed with the histochemical findings that Virchow described, but they were incorrect in assuming that it was the same material. Nevertheless, the term "amyloid," the root of which indicates amylose (i.e., starch), was retained and is now used to described amorphous accumulations of protein fibrils with a high β-sheet content.

PROTEOGLYCANS IN ALZHEIMER DISEASE

Little additional progress into the carbohydrate-protein interaction in amyloidosis was made until the late 1960's when acid mucopolysaccharides (glycosaminoglycans) were demonstrated in association amyloid fibrils [4, 5]. Liver and spleen amyloid in inflammation-associated and light chain-associated amyloidosis were associated with highly sulfated glycosaminoglycans (GAGs) (e.g., heparan sulphate) [4, 6, 7], while senile cardiac amyloidosis was associated with hyaluronic acid [8, 9]. Since these studies only assessed the glycosaminoglycan composition in amyloid deposits in end stage disease, it was unclear whether GAG deposition was concurrent with amyloid deposits or was a secondary phenomenon. Other investigators later demonstrated that deposits of highly sulfated GAGs and amyloid protein was concurrent in experimentally induced amyloidosis [10] and suggested that highly sulfated GAGs were specifically related to the pathogenesis in a variety of amyloidoses including Alzheimer's disease [11]. Ultrastructural studies further suggested a close association between AA amyloid fibrils and heparan sulfate proteoglycans [12].

Not surprisingly, histochemical studies have shown that heparan sulfate colocalizes with amyloid-β, consistent with lectin binding studies demonstrating individual components of GAGs units such as N-acetyl-D-glucosamine [13], and immunohistochemical localization of heparan sulfate proteoglycan core protein to neuritic plaques and cerebrovascular amyloid deposits in brains of AD [14,15]. Additional investigations determined the presence of chondroitin sulfate [16], decorin, a dermatan sulfate proteoglycan [17] and keratan sulfate proteoglycans [18]. Interestingly, unlike HSPGs which are colocalized with amyloid firbrils throughout the senile plaques, chondroitin sulfate and dermamatan sulfate proteoglycans are confined to the periphery, thereby surrounding the plaque cores. This suggests that the different classes of GAGs may be playing different roles during the course of the disease.

Proteoglycans are not only associated with the amyloid deposits of the senile plaques as they are also found in the neurofibrillary tangles in Alzheimer's disease [19]. Moreover, both HSPGs and chondroitin sulfate are present in the neuronal inclusions of a variety of neurodegenerative diseases [20,21].

While the precise role of proteoglycans is not known, some experimental evidence suggests that they promote amyloid deposition [22], and that amyloid-β hampers heparanase-catalyzed degradation in vitro, promoting amyloid-β deposition and accumulation [23]. Other studies have suggested that sulfate moieties of GAGs may be important to amyloid-β fibrillogenesis [24] and can inhibit proteolysis of amyloid-β fibrils [25]. Sulfated GAGs are also involved in the formation of Alzheimer-like PHFs from recombinant tau protein [26] and also promote the insolubility of PHFs [27]. Importantly, at least one form of the amyloid precursor protein is itself a chondroitin sulfate proteoglycan [28].

METABOLIC COMPROMISE IN ALZHEIMER DISEASE

In light of increasing evidence of altered glucose metabolism in AD [29-31], we were interested in exploring whether excessive glucosamine as a result of hexosamine pathway activation [as occurs, for example, in diabetes mellitus [32] led to the synthesis of glucosamine polymers. Glucosamine, the basic unit of chitin and chito-saccharides, is formed from glucose via fructose and fructose-6-phosphate. High levels of glucose, and glucosamine via subsequent hexosamine pathway activation, due

to impaired glucose metabolism in AD at the cellular level, in the presence of the normal complement of cellular enzymes, might lead to the production of glucose polymers (starch) and glucosamine polymers (chitin) respectively. In a recent study, we verified the presence of glucose polymers (amylose) in the AD brain [33]. Subsequently, we turned to the issue of glucosamine and glucosamine polymers in AD by examining brain tissue from subjects with sporadic and familial early onset AD (presenilin A431E mutation) for calcofluor reactivity [34]. Calcofluor, a fluorochrome that excites on exposure to ultraviolet light, exhibits a high affinity for chitin in vivo by interacting with β1-4 linkages [35], and is a practical technique for demonstrating chitin in tissues, as chitin is a linear polymer of glucosamine in β1-4 linkage. Interestingly, it has been demonstrated in yeast that chitin cell wall assembly is altered not only by calcofluor but also Congo red [36].

CHITIN-LIKE POLYSACCHARIDES IN ALZHEIMER DISEASE

In both sporadic and familial early onset AD, plaques of all types of all types – diffuse, neuritic, cored, cotton-wool – and blood vessels affected by amyloid angiopathy, strongly labeled using calcofluor histochemistry [35,37]. The parallel between the distribution of labeling with calcofluor histochemistry and amyloid-β immunohistochemistry was striking, with the only exception that calcofluor also stained a subset of neurofibrillary tangles in addition to plaques and blood vessels with amyloid angiopathy. To confirm specificity of the calcofluor reaction for β1-4 linked glucosamine, AD tissues were subjected to chitinase (which degrades chitin to chitobiose), followed N-acetyl glucosaminidase, and found a significant decrease in calcofluor staining, indicating that degradation into individual monosaccharide units is necessary to diminish the calcofluor reaction, and confirming specificity of calcofluor in AD for chitin-like polysaccharides. This fundamental finding of co-localization of calcofluor histochemical reactivity and amyloid-β immunohistochemistry suggests that chitin or chitin-like polysaccharides comprise an integral component of amyloid deposits.

Since chitin is highly insoluble, our studies raise the question of whether protein is purely responsible for the chemical properties of amyloid, or whether chitin-like polysaccharides influence these features as well. It is noteworthy that commercial chitin stains red with Congo Red and also shows green birefringence with plane polarized light (data not shown). This, in addition to calcoflour staining of amyloid plaques and amyloid angiopathy, is consistent with the concept that chitin influences the histochemical properties of amyloid previously ascribed to protein secondary structure. It should also be noted that diffuse plaques, and cotton wool plaques of familial early onset AD, lack the characteristic filamentous amyloid by electron microscopy [38]. Likewise, calcofluor staining was more widespread than Congo red staining in the AD brain. Thus, chitin-like polysaccharides overall are more widespread than protein fibrils of classically defined amyloid, suggesting that accumulation of N-acetyl glucosamine precedes formation of typical amyloid fibrils.

Whether brain chitin accumulation provides a protective or a deleterious function remains an important unanswered question. Commercial chitin and one of its derivatives, chitosan, have applications in wound healing and enhancement of function of inflammatory cells and fibroblasts [39]; thus, chitin within the brain may stimulate inflammatory processes such as microglial cell activation and elaboration of soluble mediators of immunity, processes which are becoming increasingly apparent in AD pathogenesis [40]. Moreover, our group and others have demonstrated that amyloid plaques are an indication of neuroprotection (e.g., by transition metal binding and diminishing cytotoxicity associated with free radicals), rather than an indication of tissue injury [41-43]. Chitin, therefore, may have a reparative or protective function in the human brain by sequestering amyloid-β and associated neurotoxic metabolites.

Our studies have provided empirical evidence of the presence of chitin or chitin-like material in the AD brain, largely in association with amyloid-β

deposits. However, it is important to recognize the complexity of the process of chitin synthesis and the unanswered questions that remain. Chitin synthesis is an energy-dependent process, requiring an N-Acetylglucosaminyltransferase using the uridine diphosphate (UDP)-activated monomer as the sugar donor [44], or a chitin synthase. While no definitive mammalian chitin synthase has been identified, a potential developmental role of chitooligosaccharides in vertebrate animals has been suggested in several studies [45-47]. Hyaluronan synthase-1 (HAS1) has been shown to convert activated glucosamine to chito-oligosaccharides in vitro using murine HAS1 gene product [48]. In addition, human synovial fluid of patients with rheumatoid or osteoarthritis contains high levels of a chitinase 3-like glycoprotein [49]. Thus, under pathological conditions, HAS1 may serve as a chitin synthase, converting activated glucosamine to chitin-like polysaccharides and facilitating the process of amyloidosis.

While chitin is extracellular, its precursor, UDP-N-acetylglucosamine is intracellular. The precise mechanism whereby chitin is transport through the cell membrane and deposited in the extracellular space therefore remains to be elucidated. In this respect, however, it is interest to note the focal presence of calcofluor reactivity within neurons affected by neurofibrillary tangle formation.

GLYCATION IN ALZHEIMER DISEASE

Advanced glycation endproducts (AGEs) comprise a diverse class of irreversible posttranslational modifications, with a propensity to form crosslinks, render proteins insoluble, interact with cell surface receptors to produce deleterious biochemical effects, and generate reactive oxygen species. While AGEs have long been associated with diabetes mellitus and senile cataracts, where high concentrations of glucose and other sugar metabolites predisposes to AGE formation, more recently, we and others demonstrated a number of AGE species in neurodegenerative diseases, including Alzheimer disease (AD) [50-53] and Parkinson disease [54]. In AD, AGEs were found in parallel with pathologic lesions [55] as well as in morphologically normal neurons (Smith and Perry, unpublished), suggesting that AGEs are not only a critical factor leading to the formation of hallmark inclusions [50,55], but are also part of the early changes seen in the disease process.

The initial step in AGE formation is the nonenzymatic attachment of sugar aldehydes or ketones to the side chains of lysine, arginine, and possibly histidine. The lysine ε-amino-derived glycation product, or Schiff base, rearranges to form a more stable amino ketone intermediate known as the Amadori product. The Amadori product can then evolve to a number of structurally more complex covalently-linked AGEs by irreversible dehydration and additional crosslinking. It is not perhaps surprising that lysine-rich proteins, such as τ, neurofilament protein, and αB crystalline, target proteins rendered insoluble by AGE modification, accumulate as insoluble inclusions that represent histopathological hallmarks of disease [50,55]. The presence of the Amadori product, indicative of active glycation, can be demonstrated by its reduction to a stable epimeric mixture of 1-glycitol-lysine and 1-mannitol-lysine, known collectively as hexitol-lysine.

Immunocytochemical and biochemical studies have suggested that one particular AGE, Nε-(Carboxymethyl)lysine (CML) is the major AGE that accumulates in vivo [56-59]. Elevated serum levels of CML are detected in patients with diabetes mellitus [59] and CML is increased in the vascular tissues of diabetic rodents and humans [60-63]. Moreover, co-localization of CML with adducts derived from products of lipid peroxidation, products of 4-hydroxy-2-nonenal and malondialdehyde, supports the concept that lipid peroxidation itself, in addition to and apart from advanced glycation, triggers the formation of CML [64].

The purpose of this study was to elucidate the role of CML and hexitol-lysine in the pathogenesis of AD. Our findings of concurrent increases in both end stage modification as well as the initial Amadori products of sugar adduction, provide evidence for an active glycation process in AD.

SUMMARY

The role of carbohydrates in the pathogenesis of various amyloidoses, including AD, is only beginning to be understood. While sulfated proteoglycans may play a role in fibrillogenesis, the identification of novel chitin-like polysaccharides in the AD brain, polysaccharides that are particularly suited to protein nucleation, may in large measure explain the biochemical characteristics of amyloid in general. While further studies are necessary to determine whether chitin is protective or deleterious, the data presented here provide evidence that chitin, previously unrecognized in humans, may be intimately associated with AD, amyloidosis, and the aging process. The implications of these data in terms of treatment advances and understanding of disease pathogenesis are considerable.

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