

# Association of Corpora Amylacea Formation with Astrocytes and Cerebrospinal Fluid in the Aged Human Brain

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**Abstract** : Corpora amylacea (CA) are glycoproteinaceous inclusions that accumulate in the human brain during normal aging and neurodegenerative diseases. Although it has been suggested that the cellular sources of CA are neuronal or glial, the mechanisms underlying CA formation remain controversial.

The aim of this study was to identify the source of CA in the human brain. Sample of the human brain tissues were obtained from the cadavers. H-E stain, periodic acid-Schiff (PAS) stain, and immunohistochemistry were performed in the brain tissues. Experimental induction of CA was also performed in rats.

CA have been found in large numbers in the superficial, rather than in the deep, layer of the white matter in the lateral ventricle that is in contact with the cerebrospinal fluid (CSF) and sometimes near the blood vessels. Destroyed choroid plexi with *psammoma* bodies have been observed in the lateral ventricle of aged brains containing substantial numbers of CA. The cores of CA were mainly composed of amorphous PAS-positive materials, and glial fibrillary acidic protein-positive astrocytic processes were attached to the surface of the CA. Weak MAP2 was detected on a few CA in the gray matter such as dentate gyrus. PAS-positive CA were located on the border of the hippocampus contacting the CSF in the lateral ventricle in the cysteamine-induced CA animal model.

Taken together, main cellular source of CA is astrocytes and CA core formation may be associated with CSF in the aged human brain.

**Keywords** : Corpora amylacea, Brain, Aging, Astrocytes, Cerebrospinal fluid, Choroid plexus

## Introduction

Corpora amylacea (CA) are cytoplasmic, glycoproteinaceous inclusion bodies that accumulate in the human brain during the course of normal aging and, to a much greater extent, in Alzheimer's disease (Cisse et al. 1993), hippocampal sclerosis accompanying temporal lobe epilepsy

(Radhakrishnan et al. 2007), and other neurodegenerative conditions (Chung and Horoupian 1996). CA are also observed in the peripheral nerves and the optic nerve of the human eye (Rejdak et al. 2007, Rejdak et al. 2011). Clinically, the presence of CA should be considered in the differential diagnosis of patients presenting with brain magnetic resonance images (MRIs) suggestive of non-enhancing space-occupying lesions (Abel et al. 2010). CA, which are composed of a polyglucosan core surrounded by an ubiquitinated outer shell, exhibit periodic acid-Schiff (PAS) staining (Cisse and Schipper 1995). Although CA were first described by Purkinje more 150 years ago, the mechanisms responsible for their formation and their pathological significance remain elusive.

Although both a neuronal (Loeffler et al. 1993, Wilhel-

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mus et al. 2009) and a glial origin has been suggested (Martin et al. 1991, Cisse et al. 1993, Singhrao et al. 1994, Schipper and Cisse 1995), the exact cellular source of CA remains to be elucidated. CA are composed of a mixture of short and long polysaccharides. Centrifugation-purified CA have been reported to yield 87% hexose, 4.7% protein, and 2.5% phosphate (Sakai et al. 1969). Immunoreactivity to astrocytes (Cisse and Schipper 1995, Schipper 1998), and oligodendroglial molecules (Singhrao et al. 1994) suggests a glial influence on CA formation.

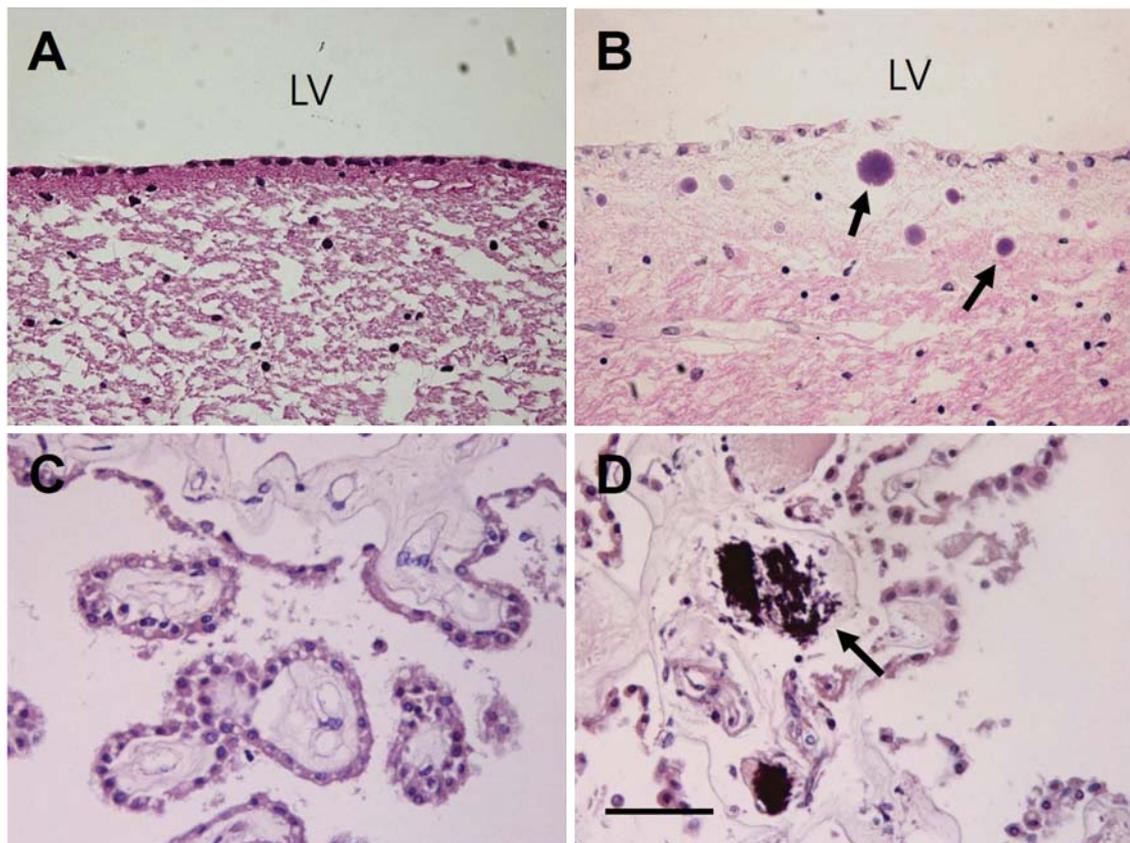
However, Maurizi (Maurizi 2010) insisted that CA form with normal aging and are usually found in brain tissue that has close contact with the cerebrospinal fluid (CSF). Thus, it seems reasonable to assume that the CA are derived from substances in CSF.

The aim of this study was to elucidate the cellular and non-cellular sources of CA in the aged human brain.

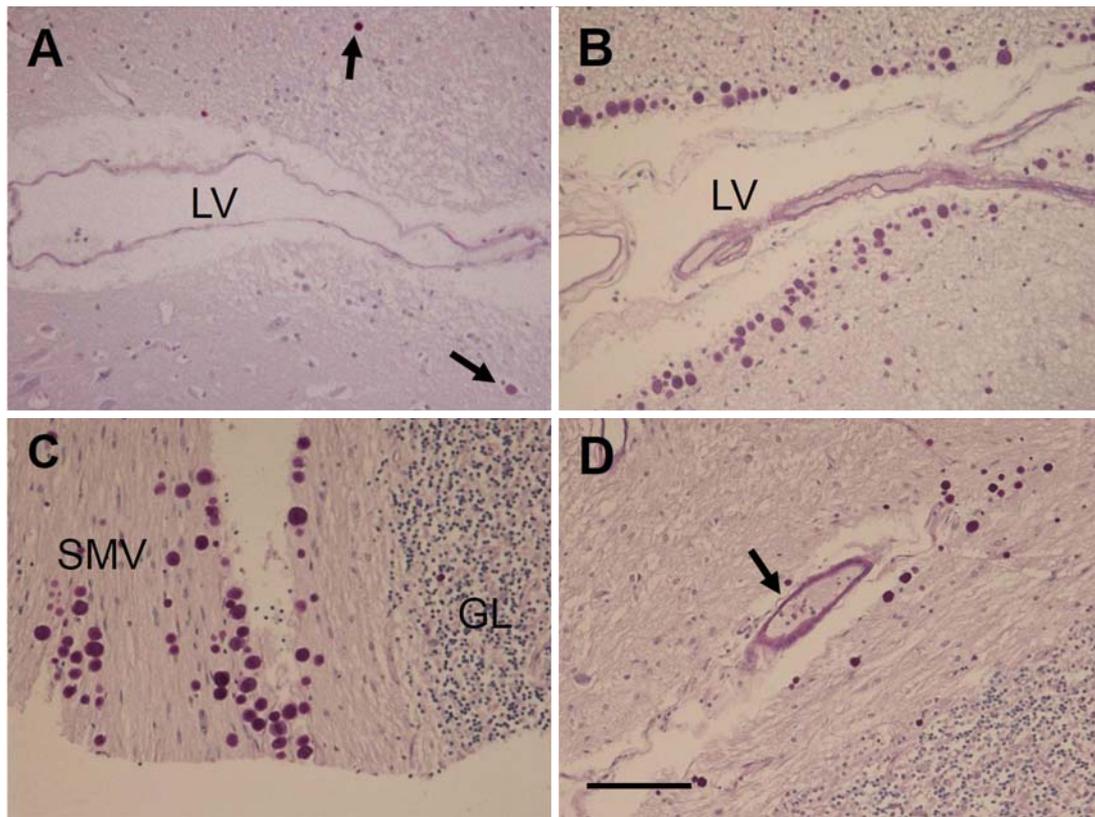
## Materials and Methods

Brain tissues, hippocampal formations, and choroid plexi in the lateral ventricle were dissected from twelve cadavers donated for medical research and education to the Department of Anatomy of the School of Medicine, Chungnam National University, Korea. We showed the data from the representative two cadavers (37- and 90-year-old males).

Experimental induction of CA was performed with the method introduced by Shipper (Schipper and Cisse 1995). Ten male Sprague-Dawley rats aged 8 weeks were purchased from Dae Han Experimental Company (Daejeon, Korea). Cytemine-HCl (CSH, Sigma Chemical Co. MO, USA) was freshly prepared on the day of administration by dissolving desiccated CSH powder in neutral saline and adjusting the pH to 7.2 with 1 M NaOH. The animals received biweekly subcutaneous injections of either CSH



**Fig. 1.** Histopathology of the hippocampus and choroid plexus in the lateral ventricle. A: The ependyma covering the hippocampus was intact in the 37-year-old male. B: The ependyma covering the hippocampus was not intact and globular substances (arrows) were located in the white matter of the hippocampus in the 90-year-old male. C: The choroid plexus in the lateral ventricle of the 37-year-old male was intact. D: The choroid plexus of the 90-year-old male was destroyed and contained *psammoma* bodies (arrow). LV, lateral ventricle. H-E stain. Scale bar=50  $\mu$ m.



**Fig. 2.** Histopathology of the region related to corpora amylacea formation. A: Few PAS-positive CA (arrows) were present in the superficial layer of the white matter of the hippocampal formation in the corner of the lateral ventricle of the 37-year-old male. B: Many PAS-positive CA were located in the superficial layer of the white matter in the corner of the lateral ventricle of the 90-year-old male. C: Many PAS-positive CA were located between the superior medullary velum (SMV) and the cerebellum of the 90-year-old male. D: PAS-positive CA were located near the blood vessel (arrow) in the SMV. PAS stain of the 90-year-old male. GL, granular layer; ML, molecular layer. Scale bar=100  $\mu$ m.

(150 mg/kg body weight,  $n=6$ ) or saline vehicle (control;  $n=4$ ) over a 6-week period. Following an additional 5-week drug washout period, the rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with 4% paraformaldehyde solution. Fixed tissues were embedded in paraffin.

Formalin-fixed tissues of the human brain were embedded in paraffin prior to hematoxylin-eosin (H-E), PAS, and immunofluorescent or immunohistochemical staining. Routine H-E and PAS staining were performed in the deparaffinized tissue sections. Some tissues were post-fixed in 2.5% glutaraldehyde solution for transmission electron microscopy.

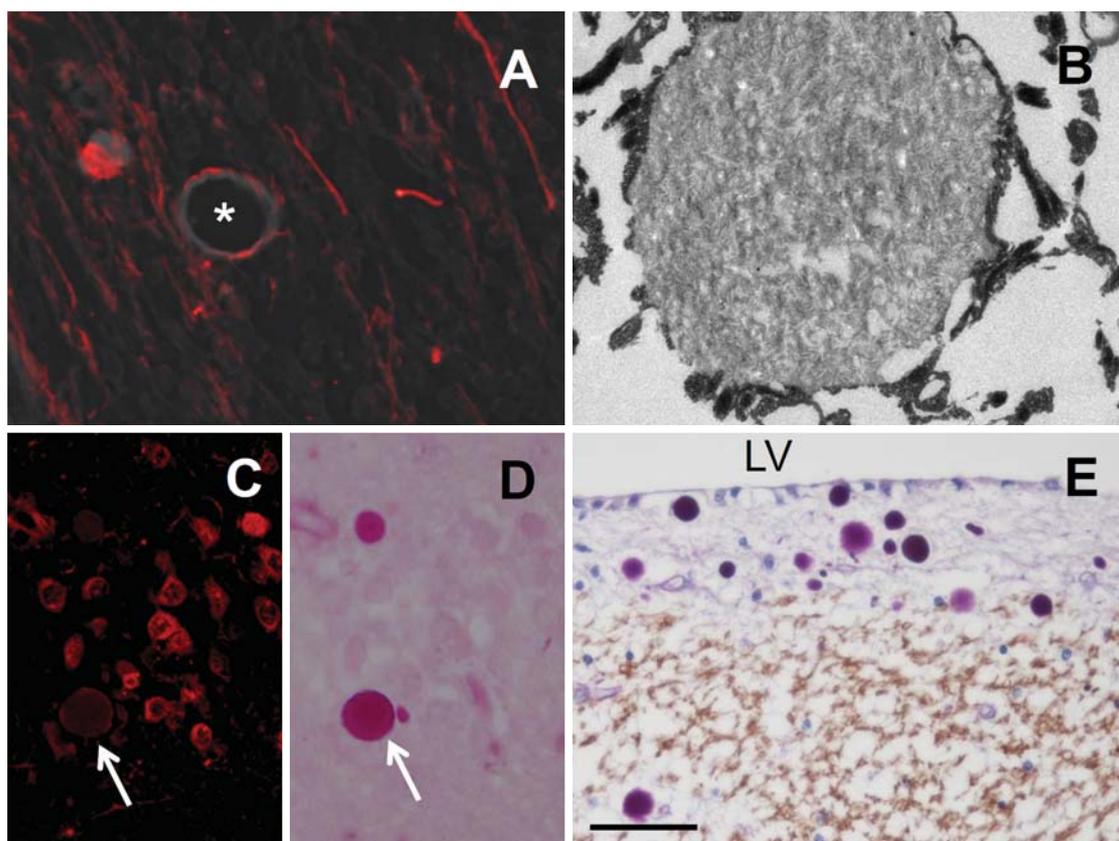
Double staining was used for glial fibrillary acidic protein (GFAP), microtubule-associated protein 2 (MAP2), and myelin basic protein (MBP) immunofluorescence or immunohistochemistry to localize CA. The deparaffinized

tissue sections were first stained with PAS, and immunofluorescence or immunohistochemistry was then performed using immunofluorescent dye or avidin-biotin complex. The antibodies used in this experiment were as follows: GFAP, polyclonal antibody, 1 : 250 (Chemicon, MA, USA); MAP2, polyclonal antibody, 1 : 500 (Novus Biologicals, CO, USA); MBP, polyclonal antibody, 1 : 250 (Chemicon, MA, USA).

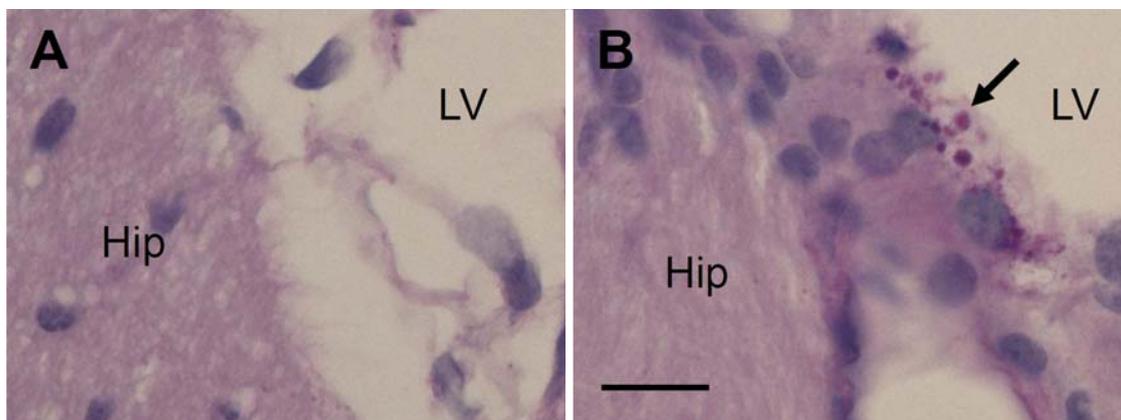
Transmission electron microscopy was performed via the usual method and observed with a transmission electron microscope (Hitachi H600, Tokyo, Japan).

## Results

The ependyma covering the hippocampus was intact, and no abnormal structure was not seen in the white matter of



**Fig. 3.** Immunofluorescent or immunohistochemical staining for GFAP, MAP2, and MBP and transmission electron microscopy of the corpora amylacea of the 90-year-old male. A: GFAP-positive processes surrounded the CA core (\*) in the hippocampal surface. B: Cellular debris attached to the CA core in the hippocampal surface. C and D: A weak MAP2-positive component (arrow) was detected on the surface of the CA. Figure D depicts a PAS-stained photograph of the same tissue presented in Figure C. E: MBP immunoreactivity was not detected on the CA in the hippocampal surface. A, C, D, double staining with PAS and immunofluorescence methods; B, transmission electron microscopy; E, double staining with PAS and immunohistochemical methods. Scale bar=10  $\mu$ m in A, 2  $\mu$ m in B, 25  $\mu$ m in C and D, 50  $\mu$ m in E.



**Fig. 4.** CA formation in the brain of the cysteamine-induced CA animal model. A: There was no PAS-positive granule in the brain of the control rats. B: Small PAS-positive granules were located on the border of the hippocampus (Hip) contacting the CSF in the lateral ventricle in the cysteamine-induced CA animal model. LV, lateral ventricle. Scale bar=20  $\mu$ m.

the hippocampus in the 37-year-old male (Fig. 1A). While the ependyma was destroyed and globular substances were found in the white matter of the hippocampus in the 90-year-old male (Fig. 1B). The choroid plexus cells of the 37-year-old male were intact (Fig. 1C), but those in 90-year-old male were destroyed (Fig. 1D). Specifically, *psammo*-ma bodies were found in the choroid plexus of the 90-year-old male (Fig. 1D).

Next, we verified the globular substances in the hippocampus with PAS stain. Few PAS-positive CA were detected in the superficial layer of the white matter in the corner of the lateral ventricle of the 37-year-old male (Fig. 2A). Although many PAS-positive CA were present in the superficial layer in contact with CSF, few CA were found in the deep layer of the corner of the lateral ventricle of the 90-year-old male (Fig. 2B). Many PAS-positive CA were also located between the superior medullary velum and the cerebellum (Fig. 2C). Sometimes, PAS-positive CA were found near the blood vessel in the aged brain (Fig. 2D). These data suggest that CA formation may be related to CSF in the ventricle or exudation from the blood vessels.

To identify origin of the CA in the hippocampus, we performed immunohistochemistry and transmission electron microscopy. The CA in the hippocampal surface of the white matter were surrounded by GFAP, an astrocyte marker, as indicated by positive results on immunofluorescence (Fig. 3A). Transmission electron microscopy showed that the CA core was composed of amorphous materials and surrounded by cellular debris (Fig. 3B). Weak MAP2, a neuronal marker, was detected on a few CA in the gray matter (e.g., the dentate gyrus) (Fig. 3C, 3D). However, MBP immunoreactivity, an oligodendrocyte marker, was not seen on the CA in the superficial layer of the hippocampus that was in contact with the lateral ventricle (Fig. 3E).

In the brain of CA induced animal model, PAS positive substance was not found in the brain of control rat (Fig. 4A). While, small PAS positive granules, probably CA, were located on the border of the hippocampus contacting the CSF in the lateral ventricle in the cysteamine-induced CA animal model (Fig. 4B).

## Discussion

CA are spherical aggregations of glucose polymers (polyglucosan or polysaccharides) (Cavanagh 1999). The CSF

contains 50-80 mg/dL of glucose and 15-45 mg/dL of protein (Felgenhauer 1974). Our study showed that most CA were localized in the superficial layer of the white matter in the lateral ventricle, which was in contact with CSF, rather than in the deep layer, and were associated with destruction of the choroid plexus. Transmission electron microscopic observation showed that the CA core was largely composed of amorphous materials, probably PAS-positive glucose polymers. Taken together, these data suggest that the formation of CA core may be associated with CSF substances (Maurizi 2010).

Many CA were present in the superficial layer of white matter in the corner of the lateral ventricle. A few CA were found on the superficial layer of the cerebral cortex near the superior sagittal sinus (data not shown). These data indicate that CA are not present on the surface of the cerebral cortex through which CSF passes, but many CA are formed in areas of stagnant CSF, for example, the corner of the lateral ventricle and other slits containing CSF.

The cellular source of CA is known to be neuronal or glial, astrocytic, or oligodendrocytic. In our study, CA were abundantly distributed in the superficial layer of white matter in the hippocampal formation near the corner of the lateral ventricle, and a few CA were found in the gray matter (e.g., the dentate gyrus). The CA on the hippocampal surface were surrounded by GFAP-positive astrocytic processes. However, the MBP-positive oligodendrocytic component was not in contact with the CA in the white matter. A weak MAP2-positive neuronal component was detected on the CA in the gray matter (e.g., the dentate gyrus). Therefore, the main cellular source of CA may be the astrocytes situated among the neural cells.

A tiny irregular lamellar fiber mass initially formed in the cytoplasm of the astrocyte with transmission electron microscopy (Leel-Ossy 2001). Where a large amount of CA developed it was practically impossible to discern the original neural structures. As the CA increased in size the normal fiber pattern of astrocytes gradually disappeared or remained only at the edge. Our data showed that the astrocytic processes of most of CA remained on the edge of the CA in the aged human brain.

Transglutaminase (TG) 1 and TG-catalyzed cross-links are associated with cytoskeletal proteins in CA in the normal aged brain and in neurodegenerative brains (Wilhelmus et al. 2009). Cross-linking by TG1 may be essential to the cross-links of the cellular components in the outer shells of

CA. In addition to TG1, CA could result from a conglomeration of interacting proteins, thrombospondin1 and ADAMTS13, from extravasated blood elements released after transient breakdown of the blood-brain barrier (Meng et al. 2009). Our data showed that the choroid plexus cells with tight junctions were destroyed in 90-year-old male, and suggested that the CSF with extravasated blood elements contribute to CA formation in the brain. Our data may be also associated with the result of a previous neuropathological study (Leel-Ossy 1995); Diabetes may enhance the tendency for forming CA by the hyperglycemia increasing the quantity of unused carbohydrate polymers, which are the main component of CA.

CSH treatment augmented the accrual of these cortical glial granules in a statistically significant manner, and the fraction of peroxidase-positive glial granules converting to mature CA in the cerebral cortex may be greater than that in other brain regions, or subcortically-derived CA are transported in centrifugal fashion towards the pial surface in the experimental model of CA (Schipper and Cisse 1995). However, our experiment showed that most of CA were found only on the border of the hippocampus, i.e., the pial surface of the lateral ventricle contacting the CSF in the cysteamine-induced CA animal model. We did not found the evidence for CA transportation in centrifugal fashion towards the pial surface from the cerebral cortex in the CA animal model.

Taken together, our data suggest that main cellular source of CA is astrocytes and CA core formation may be associated with CSF in the aged human brain.

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## 나이든 사람 뇌에서 별아교세포 및 뇌척수액과 아밀로이드소체와의 연관성

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**간추림** : 아밀로이드소체는 정상적인 노화과정이나 신경변성질환에서 뇌에 축적되는 당단백질 포함물이다. 아밀로이드소체의 세포 유래가 신경세포나 신경아교세포로 제시되고 있지만, 아밀로이드소체 형성에 대한 기전은 잘 알려져 있지 않다. 본 연구의 목적은 사람 뇌의 아밀로이드소체 유래를 확인하는데 있으며, 이를 위해 사람 뇌에서 H-E 염색, PAS 염색, 면역조직화학염색을 시행하였으며 흰쥐를 이용한 실험적 아밀로이드소체 모델도 만들었다. 아밀로이드소체는 가쪽뇌실벽 백색질의 깊은층보다 뇌척수액과 접하는 얇은층에서 관찰되었으며 가끔 혈관 주변에서도 관찰되었다. 많은 아밀로이드소체가 있는 나이든 사람 뇌에서는 맥락얼기가 파괴되어 있었으며 *psammoma*체가 관찰되었다. 아밀로이드소체 핵은 주로 무정형의 PAS 양성물질로 구성되어 있었으며, GFAP 양성인 별아교세포 돌기가 아밀로이드소체 표면에 부착되어 있었다. 치아돌기 등의 회백질에는 MAP2가 약하게 관찰되었다. Cysteamine에 의해 유도된 아밀로이드소체 동물모델에서는 PAS 양성 아밀로이드소체가 가쪽뇌실에서 뇌척수액과 접하는 해마의 경계부위에서 관찰되었다. 본 연구 결과에서 나이든 사람의 뇌에서 아밀로이드소체의 주요 세포성분은 별아교세포이며, 아밀로이드소체 핵 형성은 뇌척수액과 관련되어 있는 것을 알 수 있었다.

**찾아보기 낱말** : 아밀로이드소체, 뇌, 노화, 별아교세포, 뇌척수액, 맥락얼기