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Rapid Degeneration of Cultured Human Brain Pericytes by Amyloid \( \beta \) Protein


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Abstract: Amyloid \( \beta \) protein (A\( \beta \)) deposition in the cerebral arterial and capillary walls is one of the major characteristics of brains from patients with Alzheimer's disease and hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D). Vascular A\( \beta \) deposition is accompanied by degeneration of smooth muscle cells and pericytes. In this study we found that A\( \beta_{1-40} \) carrying the "Dutch" mutation (HCHWA-D A\( \beta_{1-40} \)) as well as wild-type A\( \beta_{1-42} \) induced degeneration of cultured human brain pericytes and human leptomeningeal smooth muscle cells, whereas wild-type A\( \beta_{1-40} \) and HCHWA-D A\( \beta_{1-42} \) were inactive. Cultured brain pericytes appeared to be much more vulnerable to A\( \beta \)-induced degeneration than leptomeningeal smooth muscle cells, because in brain pericytes cell viability already decreased after 2 days of exposure to HCHWA-D A\( \beta_{1-40} \), whereas in leptomeningeal smooth muscle cell cultures cell death was prominent only after 4–5 days. Moreover, leptomeningeal smooth muscle cell cultures were better able to recover than brain pericyte cultures after short-term treatment with HCHWA-D A\( \beta_{1-40} \). Degeneration of either cell type was preceded by an increased production of cellular amyloid precursor protein. Both cell death and amyloid precursor protein production could be inhibited by the amyloid-binding dye Congo red, suggesting that fibril assembly of A\( \beta \) is crucial for initiating its destructive effects. These data imply an important role for A\( \beta \) in inducing perivascular cell pathology as observed in the cerebral vasculature of patients with Alzheimer's disease or HCHWA-D. Key Words: Alzheimer's disease—Amyloid \( \beta \) protein—Cerebrovascular—Degeneration—Pericytes—Smooth muscle cells.


Senile plaques and cerebrovascular amyloid are two of the characteristic pathologic lesions in the brains of patients with Alzheimer's disease (Mandybur, 1975; Glenner et al., 1981; Khachaturian, 1985). The major component of these lesions is the 39–42-amino-acid amyloid \( \beta \) protein (A\( \beta \)) (Glenner and Wong, 1984a), which is formed by proteolytic cleavage of the amyloid precursor protein (APP) (Kang et al., 1987; Ponte et al., 1988). Similar deposits of A\( \beta \) are observed in brains of patients with Down's syndrome and hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D) (Glenner and Wong, 1984b; Luyendijk et al., 1988). The latter disease is caused by a mutation at amino acid 22 of the A\( \beta \) sequence (Levy et al., 1990), resulting in the formation of extensive cerebrovascular amyloid, often leading to fatal hemorrhages.

Cerebrovascular A\( \beta \) deposition is observed in leptomeningeal arteries as well as in cortical arterioles and capillaries. An important role in the production of cerebrovascular A\( \beta \) has been assigned to smooth muscle cells (SMCs) because APP and A\( \beta \) have been immunohistochemically identified in or adjacent to these cells (Shoji et al., 1990; Tagliavini et al., 1990; Yamaguchi et al., 1992; Frackowiak et al., 1994; Wisniewski and Wegiel, 1994), and cultured SMCs have been shown to produce and secrete APP (Van Nostrand et al., 1994). Besides, SMCs in amyloid-laden vessels are subject to degeneration, suggesting a toxic effect of amyloid or A\( \beta \) (Kawai et al., 1993; Wisniewski and Wegiel, 1994).

In a previous report, the toxicity of A\( \beta_{1-42} \), but not the shorter isoform A\( \beta_{1-40} \), for cultured leptomeningeal SMCs has been described (Davis-Salinas et al., 1995). Furthermore, replacing glutamic acid at position 22 of A\( \beta \) by glutamine, as in HCHWA-D (Levy et al., 1990), caused a remarkable reversal in the toxic
effects of the Aβ peptides: HCHWA-D Aβ1–40, but not HCHWA-D Aβ1–42, caused degeneration of cultured SMCs (Davis and Van Nostrand, 1996). Aβ-induced degeneration of cultured SMCs was accompanied by an increase in APP and Aβ production (Davis-Salinas and Van Nostrand, 1995; Davis-Salinas et al., 1995), suggesting a self-amplifying process of Aβ and APP production and cellular degeneration.

Deposition of Aβ also occurs in capillaries of the cortex of brains from Alzheimer’s disease patients. Pericytes are a ubiquitous and prominent cell type of microvessels, located in a periendothelial position (Rhodin, 1980; Diaz-Flores et al., 1991). Their phenotypic relation with SMCs and their position close to microvessels, located in a periendothelial cortex of brains from Alzheimer’s disease patients, suggesting a self-amplifying process of Aβ. Besides, pericytes in amyloid-containing capillaries are prone to degeneration (Wisniewski et al., 1992). Previously, we described the isolation and characterization of human brain pericytes (HBPs) (Verbeek et al., 1994) and SMCs (Van Nostrand et al., 1994) from human autopsy tissue. In this study we describe the effects of various synthetic Aβ peptides on the degeneration of these cell types and their APP metabolism. We show that HBPs are more vulnerable to, and have a lower capacity to recover from, Aβ-induced degeneration than SMCs. Furthermore, in both cell types, cellular degeneration is preceded by an increase in APP production, and both these processes are probably mediated by assembly of Aβ.

MATERIALS AND METHODS

Materials

The Aβ peptides used in this study were synthesized and characterized as described previously (Davis-Salinas et al., 1995). The following peptides were used: Aβ1–40, Aβ1–42, HCHWA-D Aβ1–40, and HCHWA-D Aβ1–42. The HCHWA-D peptides contain a glutamine at amino acid 22 instead of a glutamic acid. Lyophilized peptides were dissolved in sterile water at 250 µM.

Cell culture

HBPs and human leptomeningeal SMCs (HLSMCs) were isolated and characterized as described previously (Van Nostrand et al., 1994; Verbeek et al., 1994, 1995). Cells were maintained in Eagle’s modification of essential medium supplemented with 10% human serum (Gemini BioProducts, Calabasas, CA, U.S.A.), 10% newborn calf serum (Life Technologies), 150 µg/ml endothelial cell growth factor, and antibiotics. For degeneration experiments, triplicate wells with cultured cells were preincubated with Eagle’s modification of essential medium containing 0.1% bovine serum albumin, 1 µg/ml hydrocortisone, 20 ng/ml insulin-like growth factor, and antibiotics (serum-free medium) for 4 h. Subsequently, cells were incubated with fresh serum-free medium, supplemented with synthetic Aβ peptides at 25 µM, for 6–12 days. Cells were routinely inspected and photographed using an Olympus phase-contrast microscope. Cell viability was quantified using a fluorescence live/dead cell assay according to the manufacturer’s description (Molecular Probes). The cultures were examined using an Olympus fluorescence microscope, and the percentage of dead cells was determined from at least two counts per well.

Western blotting analysis

After incubation culture supernatant was collected and diluted 1:1 with nonreducing sample buffer. Cells were washed once with phosphate-buffered saline and then solubilized in the wells with lysis buffer [50 mM Tris-HCl, 150 mM NaCl (pH 7.5), 1% sodium dodecyl sulfate (SDS), 5 mM EDTA, 500 µM 4-(2-aminoethyl)benzenesulfonfluoride, 10 µg/ml leupeptin, and 10 µg/ml chymostatin] for 15 min. After centrifugation at 14,000 g for 10 min, the protein content of diluted samples of the resulting supernatant was determined according to the method of Bradford (1976). Equal protein amounts were loaded and fractionated on nonreducing 10% SDS-polyacrylamide gels and subsequently electrophoretically transferred to Hybond nitrocellulose membranes (Amersham Corp.) in blotting buffer [25 mM Tris-HCl (pH 8.6), 192 mM glycine, and 20% methanol]. Blots were washed for 15 min in phosphate-buffered saline containing 0.05% Tween-20, preincubated with blocking solution (5% lowfat milk powder in PBST), washed three times with PBST, and subsequently incubated with anti-APP MAb P2-1 (Van Nostrand et al., 1989) and peroxidase-labeled sheep anti-mouse antibodies (Amersham). Detection was performed by chemiluminescence according to the manufacturer’s description (Amersham) and exposure to Kodak X-OMAT-R films. Signal intensities were quantified by scanning laser densitometry.

RESULTS

Synthetic Aβ peptides were applied to cultured HBPs and tested for their effects on morphology. Treatment of cultured HBPs with HCHWA-D Aβ1–40 (25 µM) for 6 days clearly induced signs of cellular degeneration. Cell contours disappeared, indicating a disruption of the cell membrane, but all cells remained attached to the culture dish (Fig. 1). Similar, but less robust, effects on the morphology were observed with wild-type Aβ1–42 (25 µM, 6 days; data not shown). In contrast, incubation with either wild-type Aβ1–40 or HCHWA-D Aβ1–42 did not affect cell morphology (data not shown). Shortly after application, the HCHWA-D Aβ1–42 peptide formed aggregates in solution, which was not observed with the other peptides. HCHWA-D Aβ1–40 was more potent in inducing cellular degeneration than wild-type Aβ1–42; therefore, in the following experiments we concentrated on the effects of the HCHWA-D Aβ1–40 peptide only.

Addition of HCHWA-D Aβ1–40 to HBP cultures strongly reduced the viability of the cells as determined by counting the numbers of live and dead cells in the cultures after 6 days, whereas, in accordance with the microscopic inspections, treatment with Aβ1–40 or HCHWA-D Aβ1–42 did not affect cell viability (Fig. 2). The effects of synthetic Aβ peptides on HLSMC cultures, treated in parallel experiments, were largely comparable to the effects on HBP cultures (data not shown). Visual inspection of the HLSMC and HBP cultures during a 6-day treatment with wild-type Aβ1–42 or HCHWA-D Aβ1–40 revealed that degenera-
The rapid degradation of HSPGs in the presence of HCMV, as shown in the figure, suggests that HSPGs play a role in the control of the cell cycle. This degradation is mediated by the viral protein gp155, which targets HSPGs for degradation.

**Figure 1:** Phase contrast micrographs of control and HCMV-infected HEPG cells (a) control medium (b) medium containing HCMV.

**Figure 2:** Number of cells in HEPG cultures after treatment with HCMV-A2/4.

**Figure 3:** Controls and experiments with HCMV-A2/4.

The following equations are used to calculate the difference in cell counts:

\[ \text{Difference} = \text{Control} - \text{Experimental} \]

The results of these calculations are presented in the table below:

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Control</th>
<th>Experimental</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>10000</td>
<td>8000</td>
</tr>
<tr>
<td>2</td>
<td>9900</td>
<td>7000</td>
</tr>
<tr>
<td>4</td>
<td>9800</td>
<td>6000</td>
</tr>
<tr>
<td>6</td>
<td>9700</td>
<td>5000</td>
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The decrease in cell counts is significant after 2 hours of exposure to HCMV-A2/4.
Induction of cellular and secreted APP in HBP cultures after Aβ treatment. **Top:** Immunoblot analysis of cellular (lanes 1 and 2) and secreted (lanes 3 and 4) APP in cultured HBPs incubated with control medium (lanes 1 and 3) or medium containing HCHWA-D Aβ1-40 (lanes 2 and 4). Sizes of molecular mass markers are indicated on the right in kDa. **Bottom:** Semi-quantitative densitometric analysis of the effects of various Aβ peptides on the levels of cellular and secreted APP in cultured HBPs. Data are mean ± SD (bars) values of representative experiments. Statistical analysis was performed using Student's t test. The level of significance of the difference from control values is indicated as follows: ***p < 0.001, *p < 0.05, p = 0.05 is not indicated. D Aβ1-40, HCHWA-D Aβ1-40; D Aβ1-42, HCHWA-D Aβ1-42.

In this report we described the effects of synthetic Aβ peptides on cultured HBPs as a model for Aβ-induced cellular degeneration of the capillary wall. HCHWA-D Aβ1-40, but not the longer isoform HCHWA-D Aβ1-42, induced degeneration of cultured HBPs, with a concomitant increase in cellular and secreted APP levels. Similar, but less robust, degenerative effects were observed with wild-type Aβ1-42 but not with Aβ1-40. These results are in agreement with several studies describing the effects of synthetic Aβ peptides on cultured HLSMCs. Both HCHWA-D Aβ1-40 and Aβ1-42, when added in a soluble form, similar to this study, caused degeneration of HLSMCs (Davis-Salinas et al., 1995; Davis and Van Nostrand, 1996). In contrast, preaggregation of Aβ entirely abol-

number of viable cells in culture, APP expression further increased between day 4 and 6 of the 6-day HCHWA-D Aβ1-40 treatment period.

In parallel experiments, HBPs and HLSMCs were treated with HCHWA-D Aβ1-40 for 2 or 4 days and subsequently incubated in control medium for 4 or 2 days, respectively, to test for their recovery capacity. During this second phase of incubation, cell viability of both HBP and HLSMC cultures further decreased (compare Fig. 6A and 7A; e.g., 36% dead HBPs in Fig. 7A and 21% dead HBPs in Fig. 6A after 2 days of HCHWA-D Aβ1-40 treatment with or without recovery period, respectively), indicating that the adverse effect on viability could develop after removal of the toxic peptide. Short exposure (2 or 4 days) to HCHWA-D Aβ1-40 followed by a period in control medium is sufficient to induce maximal cell death over a 6-day period in HBP cultures. Morphological examination of HLSMC cultures revealed that these cells were able to recover after such short-term (2–4 days) exposure to HCHWA-D Aβ1-40; this was confirmed by determination of the number of dead cells that remained below maximal values (Fig. 7A). In HLSMC cultures, a strong increase in the number of dead cells required an exposure to the toxic peptide for 4–6 days.

Cell death in HBP cultures already reached plateau values when they were treated with HCHWA-D Aβ1-40 for 2 or 4 days and counted at day 6. However, when APP levels of either HBP or HLSMC cultures were compared in the case of treatment for 2 or 4 days with HCHWA-D Aβ1-40 and direct analysis (Fig. 6B) or harvested after a recovery period (Fig. 7B), the expression of APP was lower in the latter population, indicating a direct and reversible effect of Aβ on APP expression, in contrast to the effect on cell viability, which was more delayed.

**DISCUSSION**

In this report we described the effects of synthetic Aβ peptides on cultured HBPs as a model for Aβ-induced cellular degeneration of the capillary wall. HCHWA-D Aβ1-40, but not the longer isoform HCHWA-D Aβ1-42, induced degeneration of cultured HBPs, with a concomitant increase in cellular and secreted APP levels. Similar, but less robust, degenerative effects were observed with wild-type Aβ1-42 but not with Aβ1-40. These results are in agreement with several studies describing the effects of synthetic Aβ peptides on cultured HLSMCs. Both HCHWA-D Aβ1-40 and Aβ1-42, when added in a soluble form, similar to this study, caused degeneration of HLSMCs (Davis-Salinas et al., 1995; Davis and Van Nostrand, 1996). In contrast, preaggregation of Aβ entirely abol-

![FIG. 3.](image-url) Induction of cellular and secreted APP in HBP cultures after Aβ treatment. **Top:** Immunoblot analysis of cellular (lanes 1 and 2) and secreted (lanes 3 and 4) APP in cultured HBPs incubated with control medium (lanes 1 and 3) or medium containing HCHWA-D Aβ1-40 (lanes 2 and 4). Sizes of molecular mass markers are indicated on the right in kDa. **Bottom:** Semi-quantitative densitometric analysis of the effects of various Aβ peptides on the levels of cellular and secreted APP in cultured HBPs. Data are mean ± SD (bars) values of representative experiments. Statistical analysis was performed using Student's t test. The level of significance of the difference from control values is indicated as follows: ***p < 0.001, *p < 0.05, p = 0.05 is not indicated. D Aβ1-40, HCHWA-D Aβ1-40; D Aβ1-42, HCHWA-D Aβ1-42.

![FIG. 4.](image-url) Effects of exposure to HCHWA-D Aβ1-40 (D Aβ1-40) and Congo red on the viability of HBP cultures after 6 or 12 days of incubation. Congo red entirely inhibited the degenerative effects of D Aβ1-40. Data are mean ± SD (bars) values of three or more counts in a representative experiment. Statistical analysis was performed as described in the legend to Fig. 2.
RAPID DEGENERATION OF PERICYTES BY Aβ

FIG. 5. Effects of HCHWA-D Aβ40 and Congo red on cellular APP levels in HBP cultures. Top: Immunoblot analysis of cellular APP levels in cultured HBP incubated with control medium (lane 1), Congo red (25 µM; lane 2), HCHWA-D Aβ40 (25 µM; lane 3), or HCHWA-D Aβ1-40 and Congo red simultaneously (lane 4). Sizes of molecular mass markers are indicated on the right in kDa. Bottom: Semiquantitative densitometric analysis of the effects of incubation with HCHWA-D Aβ1-40 (D Aβ1-40) and Congo red on the levels of cellular APP in cultured HBPs. Data are mean ± SD (bars) values of representative experiments. Statistical analysis was performed as described in the legend to Fig. 2.

FIG. 6. Effect of short-term incubation with HCHWA-D Aβ1-40 (0, 2, 4, or 6 days) on (A) viability and (B) cellular APP levels of HBP and SMC cultures. Data are mean ± SD (bars) values of a representative experiment. Statistical analysis was performed using Student's t test. The level of significance of the difference from control values is indicated as follows: """"p < 0.001, """"p < 0.01, """"p < 0.05. p ≥ 0.05 is not indicated. The absolute levels of APP expression (as shown in B) may vary between the experiments and between individual HBP and HLSMC cultures.

boring parenchyma (Scholz, 1938; Morel and Wildi, 1952). As cultured HBPs are more sensitive to Aβ treatment and as capillaries only contain one layer of pericytes, in contrast to arteries, which contain multiple layers of SMCs, Aβ-induced degeneration of pericytes may cause capillaries to disintegrate more easily than arteries, after which the amyloid may start to penetrate into the brain parenchyma, resulting in dyshoric angiopathy.

Our data also provided more insight into the dynamics of Aβ-induced cellular degeneration and the induction of APP production. The increase in cellular APP production is directly related to the presence of active Aβ isoforms, such as HCHWA-D Aβ1-40, because short-term incubation induced an immediate increase in APP production and, after removal of the peptides, APP production decreased again. An increase in APP production may also involve increased production of endogenous Aβ (Davis-Salinas et al., 1995) and of C-terminal fragments of APP (authors' unpublished data).

lished the effect on cultured HLSMCs (Davis-Salinas and Van Nostrand, 1995), whereas preaggregated Aβ was more toxic to neuronal cell cultures than nonaggregated Aβ (Yankner et al., 1990; Pike et al., 1991, 1993). These data indicate that related cell types, such as HBPs and HLSMCs, respond similarly to Aβ treatment but differ from neuronal cells in their response to Aβ.

However, quantitative differences could be observed in the effects of Aβ on cultured HBPs or HLSMCs. In cultured HBPs, in contrast to cultured HLSMCs, treatment with HCHWA-D Aβ1-40 resulted in increased levels of secreted soluble APP, which may relate to subtle differences in response of the different cell types. Furthermore, cultured HBPs were more vulnerable to HCHWA-D Aβ1-40 treatment than cultured HLSMCs. This observation may provide an explanation for the phenomenon of dyshoric angiopathy in Alzheimer's disease brains, defined as fine radiating deposits of amyloid extending from amyloid-laden cortical capillaries and small arterioles into the neighbor-
assembly of peptide, cell viability continued to decrease for several days, indicating that the intracellular processes leading to cell death could not be reversed anymore.

The mechanisms leading to Aβ-induced cell death are not clarified yet, but the experiments with Congo red point to a mechanism in which Aβ assembly may be the crucial factor similar to what has been described for the neurotoxic effects of Aβ (Lorenzo and Yankner, 1994). Congo red inhibits neurotoxicity of Aβ either by binding to preformed fibrils or, alternatively, by inhibiting fibril formation (Lorenzo and Yankner, 1994). We also found that Congo red protected cultured HBPs against cellular degeneration by HCHWA-D Aβ1-42, suggesting that Aβ assembly is a common mechanism in the destructive effects of Aβ on various cell types. If assembly of Aβ is necessary for its toxicity, the aggregation rate of each Aβ isoform may be decisive for its effect. Wild-type Aβ1-42 fibrillizes faster than Aβ1-40 (Jarrett et al., 1993), and in accordance with this, wild-type Aβ1-42 is toxic for cultured HBPs and SMCs, whereas Aβ1-40 is not. Furthermore, Aβ peptides containing the Dutch mutation have been described to assemble into fibrils more rapidly than wild-type Aβ (Wisniewski et al., 1991; Clements et al., 1993). In line with these observations, HCHWA-D Aβ1-40 causes degeneration of cultured HBPs and SMCs, whereas wild-type Aβ1-40 is nonactive. In analogy, it would be anticipated that HCHWA-D Aβ1-42 is even more toxic than HCHWA-D Aβ1-40. However, HCHWA-D Aβ1-42 is not cytotoxic for HBP or HLSMC cultures, which may be related to the observation that this peptide is the only one that very rapidly formed aggregates in solution, as was recently reported (Davis and Van Nostrand, 1996). Similar to the previous description of the absence of cellular degeneration after addition of preaggregated Aβ1-42 to cultured HLSMCs (Davis-Salinas and Van Nostrand, 1995), these aggregates of HCHWA-D Aβ1-42 are not toxic for cultured HBPs. Although the protective effect of Congo red suggests that assembly of Aβ is essential for its cytotoxic effect on cultured HBPs, interaction with the cell surface during assembly seems to be crucial too, as Aβ assembly in solution alone does not induce cellular degeneration.

In conclusion, Aβ-induced cellular degeneration seems to be a cell-specific event, that, even in closely related cell types such as HBPs and HLSMCs, may result in different responses. Future study of the mechanisms of Aβ-induced degeneration of cultured HBPs and HLSMCs may provide more insight into the mechanisms of cellular degeneration that is observed in Alzheimer’s disease and HCHWA-D brains. A common mechanism in Aβ-induced cytotoxicity may be the formation of Aβ fibrils during interaction of Aβ with target cells, although this needs further investigation. The data in this study support the important role for Aβ in causing vascular pathology as observed in Alzheimer’s disease and HCHWA-D brains and indicate that cultured human cerebrovascular cells may serve as a model for the study of vascular amyloid formation, which will allow for future preclinical testing of anti-amyloidogenic drugs.

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