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Rapid Degeneration of Cultured Human Brain Pericytes by Amyloid β Protein

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Abstract: Amyloid β protein (Aβ) 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 Aβ 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 Aβ-induced degeneration than leptomeningeal smooth muscle cells, because in brain pericyte cultures 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 Aβ is crucial for initiating its destructive effects. These data imply an important role for Aβ 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 β protein—Cerebrovasculature—Degeneration—Pericytes—Smooth muscle cells.
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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 β protein (Aβ) (Glenner and Wong, 1984*a*), which is formed by proteolytic cleavage of the amyloid precursor protein (APP) (Kang et al., 1987; Ponte et al., 1988). Similar deposits of Aβ are observed in brains of patients with Down's syndrome and hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D) (Glenner and Wong, 1984*b*; Luyendijk et al., 1988). The latter disease is caused by a mutation at amino acid 22 of the Aβ sequence (Levy et al., 1990), resulting in the formation of extensive cerebrovascular amyloid, often leading to fatal hemorrhages.

Cerebrovascular Aβ deposition is observed in leptomeningeal arteries as well as in cortical arterioles and capillaries. An important role in the production of cerebrovascular Aβ has been assigned to smooth muscle cells (SMCs) because APP and Aβ 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 Aβ (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 Aβ by glutamine, as in HCHWA-D (Levy et al., 1990), caused a remarkable reversal in the toxic

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Abbreviations used: Aβ, amyloid β protein; APP, amyloid precursor protein; HBP, human brain pericyte; HCHWA-D, hereditary cerebral hemorrhage with amyloidosis-Dutch type; HLSMC, human leptomeningeal smooth muscle cell; PBST, phosphate-buffered saline containing 0.05% Tween-20; SDS, sodium dodecyl sulfate; SMC, smooth muscle cell.

effects of the Aβ peptides: HCHWA-D $A\beta_{1-40}$, but not HCHWA-D $A\beta_{1-42}$, 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 microvascular Aβ deposits suggest that pericytes may be involved in amyloid formation in cerebral capillaries. 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: $A\beta_{1-40}$, $A\beta_{1-42}$, HCHWA-D $A\beta_{1-40}$, and HCHWA-D $A\beta_{1-42}$. 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 fluorescent 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)benzenesulfonyl fluoride, 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 $A\beta_{1-40}$ (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 morphology were observed with wild-type $A\beta_{1-42}$ (25 μM, 6 days; data not shown). In contrast, incubation with either wild-type $A\beta_{1-40}$ or HCHWA-D $A\beta_{1-42}$ did not affect cell morphology (data not shown). Shortly after application, the HCHWA-D $A\beta_{1-42}$ peptide formed aggregates in solution, which was not observed with the other peptides. HCHWA-D $A\beta_{1-40}$ was more potent in inducing cellular degeneration than wild-type $A\beta_{1-42}$; therefore, in the following experiments we concentrated on the effects of the HCHWA-D $A\beta_{1-40}$ peptide only.

Addition of HCHWA-D $A\beta_{1-40}$ 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 $A\beta_{1-40}$ or HCHWA-D $A\beta_{1-42}$ 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 $A\beta_{1-42}$ or HCHWA-D $A\beta_{1-40}$ revealed that degenera-

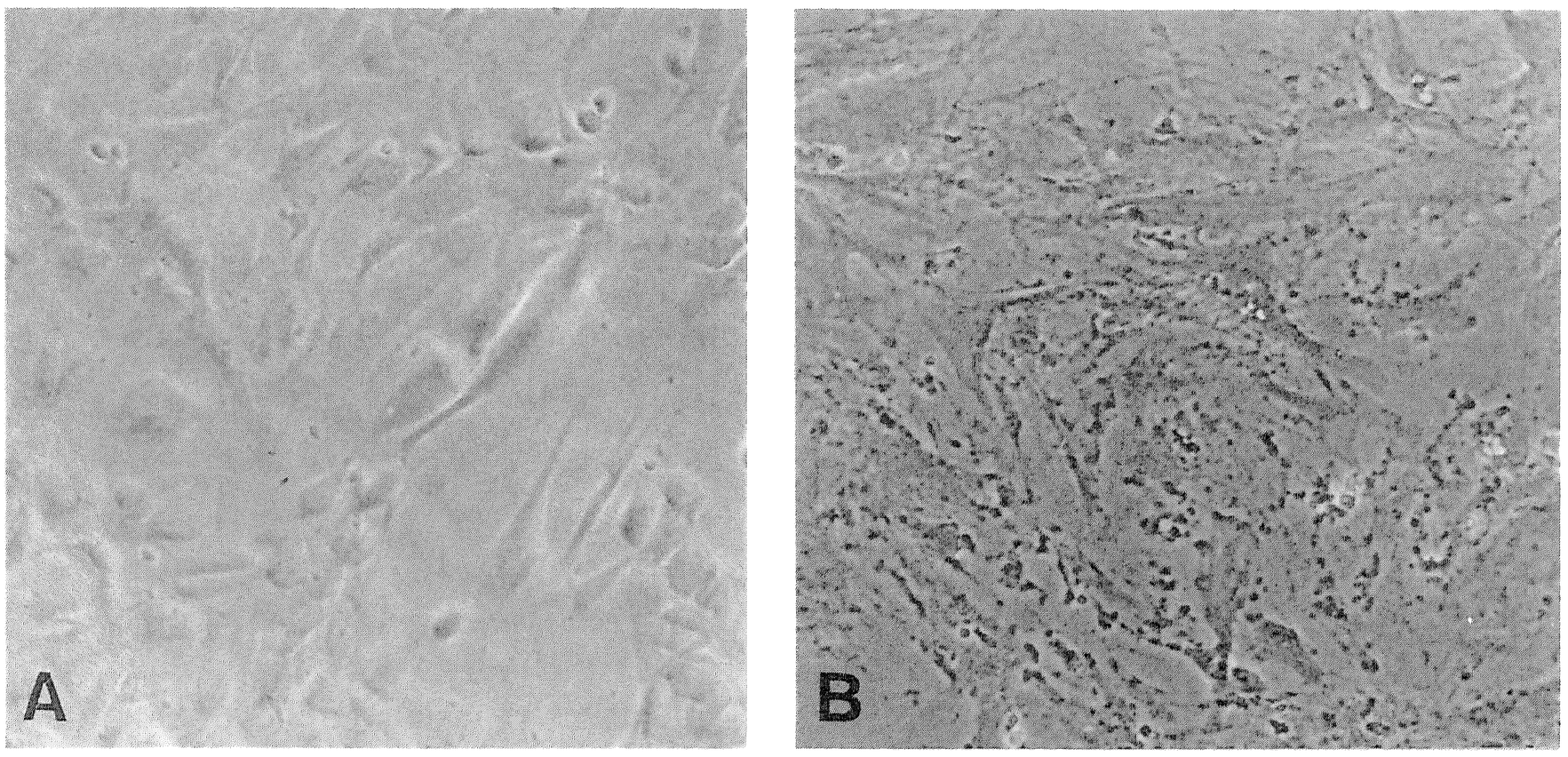

FIG. 1. Phase-contrast micrographs of cultured HBPs treated for 6 days with (**A**) control medium or (**B**) medium containing HCHWA-D $A\beta_{1-40}$ (25 μM). Treatment with HCHWA-D $A\beta_{1-40}$ caused degeneration of the cells.

tion of cultured HBPs already started after 1–2 days, whereas degeneration of HLSMC cultures was postponed to 4–5 days after the start of the incubation (see below).

Because APP levels are increased in vessels affected by $A\beta$ deposition, we analyzed the possibility of $A\beta$-induced APP expression in HBP cultures. Indeed, both cellular and secreted APP levels were strongly increased after 6 days of treatment with HCHWA-D $A\beta_{1-40}$, but not with $A\beta_{1-40}$ or HCHWA-D $A\beta_{1-42}$ (Fig. 3).

Because it has been described that the amyloid-binding dye Congo red could abolish the neurodegenerative effects of $A\beta_{1-40}$, we tested its ability to compensate for the degenerative effects of HCHWA-D $A\beta_{1-40}$ treatment on HBP cultures. When these cells were incubated simultaneously with HCHWA-D $A\beta_{1-40}$ and Congo red during 6–12 days of incubation in a 1:1 or 5:1 molar ratio, cellular degeneration was completely absent, and cell death was reduced to control levels (Fig. 4). Incubation with Congo red alone had no visible effects on the morphology of the cells but seemed to augment cellular survival as seen by the slight, but reproducible, reduction in the number of dead cells in control cultures. Similar effects were observed with HLSMC cultures (data not shown). Furthermore, the increase in both cellular (Fig. 5) and secreted (data not shown) APP by HBPs after stimulation with HCHWA-D $A\beta_{1-40}$ was entirely blocked by Congo red.

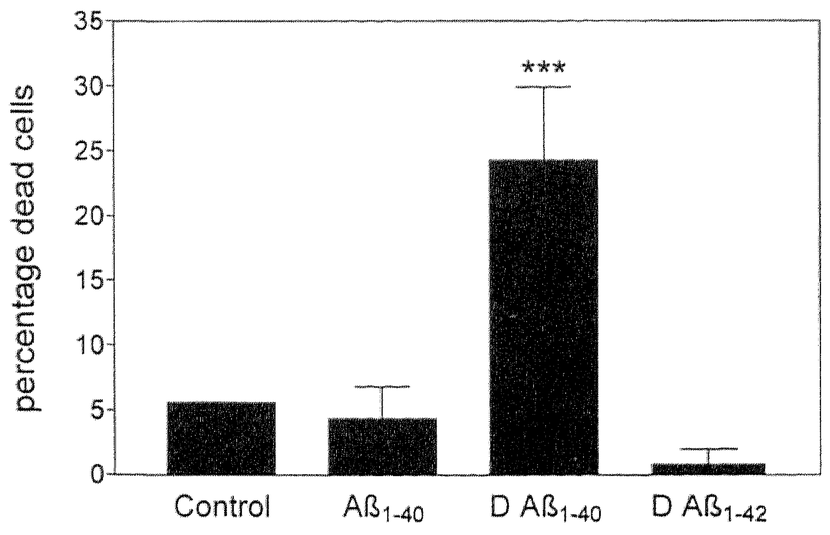

FIG. 2. Number of dead cells in HBP cultures after treatment for 6 days with $A\beta$ peptides. Data are mean ± SD (bars) values of three or more counts in a representative experiment. Statistical analysis was performed using Student's *t* test. The level of significance of the difference from control values is indicated as follows: $^{***}p < 0.001$. $p \geq 0.05$ is not indicated. D $A\beta_{1-40}$, HCHWA-D $A\beta_{1-40}$; D $A\beta_{1-42}$, HCHWA-D $A\beta_{1-42}$.

As noted above, HBP and HLSMC cultures responded similarly to $A\beta$ treatment, but HBP cultures invariantly contained a higher percentage of dead cells than HLSMC cultures (5 and <1%, respectively; data not shown), and they seemed to respond faster to $A\beta$ treatment than HLSMC cultures, suggesting that they were more sensitive to degeneration. Therefore, we investigated the time dependence of HCHWA-D $A\beta_{1-40}$ incubation and investigated the capacity of HBPs and HLSMCs to recover from short-term exposure to this peptide. Cultures of either cell type were incubated for 0, 2, 4, or 6 days with HCHWA-D $A\beta_{1-40}$ after incubation for 6, 4, 2, or 0 days, respectively, in control medium and were harvested at day 6. From Fig. 6A it can be seen that already after 2 days of HCHWA-D $A\beta_{1-40}$ treatment the viability of HBP cultures was significantly reduced, and the number of dead cells increased until day 6 of the incubation, whereas the viability of HLSMC cultures remained high until day 4 and was only significantly reduced after 6 days of exposure, in line with the microscopic observations of morphological degeneration. The pattern of induction of APP expression was similar in either HBP or HLSMC cultures (Fig. 6B) but differed in timing from the effects on viability. An increase was already observed after 2 days of incubation of either cell type and reached maximal levels after 4 days. The overall APP levels were slightly reduced after 6 days of incubation, but when the APP levels are corrected for the

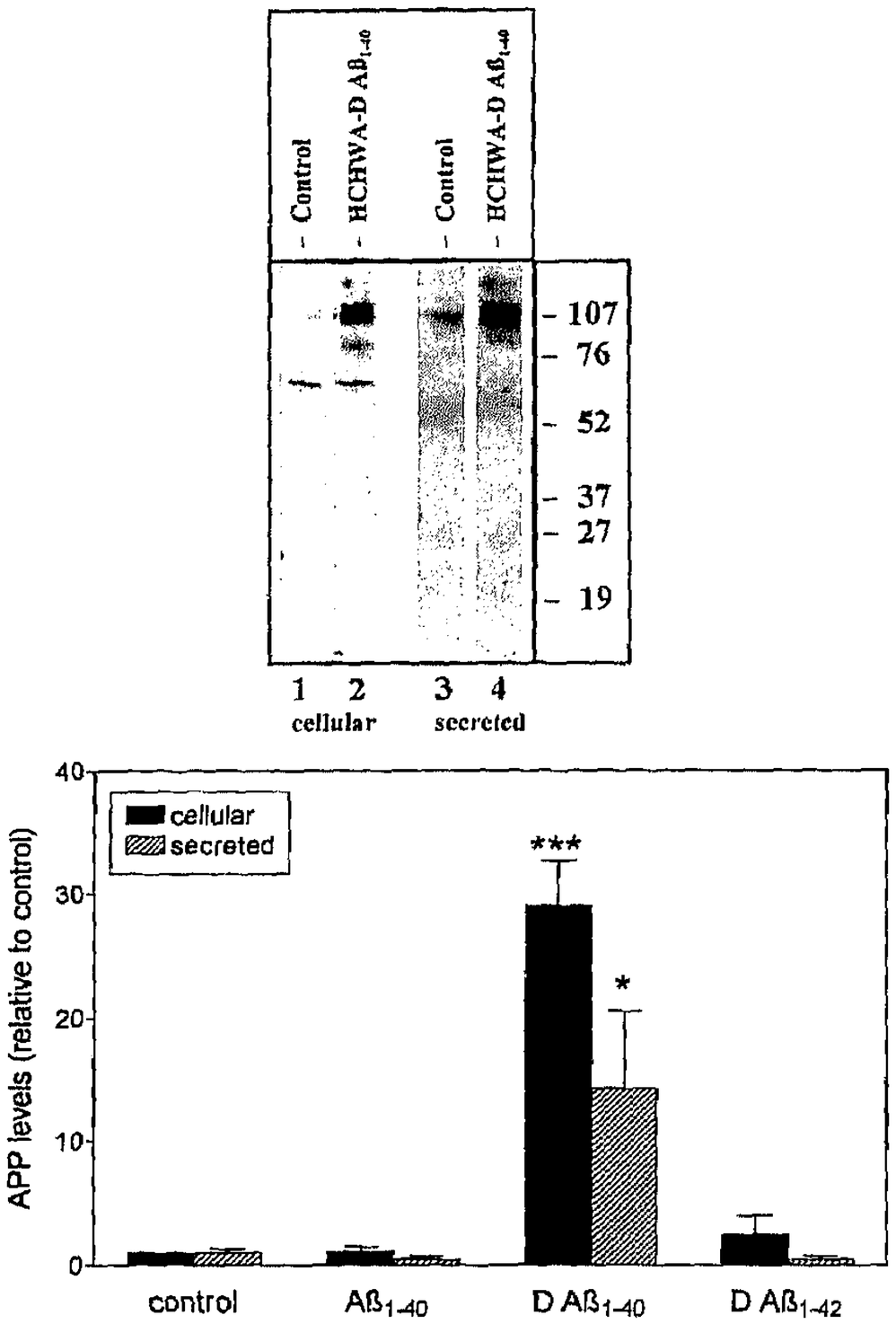

FIG. 3. Induction of cellular and secreted APP in HBP cultures after Aβ treatment. **Top:** Immunoblot analysis of cellular (lanes 1 and 2) and secreted (lanes 3 and 4) APP in cultured HBPs incubated with control medium (lanes 1 and 3) or medium containing HCHWA-D Aβ_{1-40} (lanes 2 and 4). Sizes of molecular mass markers are indicated on the right in kDa. **Bottom:** Semiquantitative densitometric analysis of the effects of various Aβ peptides on the levels of cellular and secreted APP in cultured HBP. 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: $^{***}p < 0.001$, $^{*}p < 0.05$. $p \geq 0.05$ is not indicated. D Aβ_{1-40}, HCHWA-D Aβ_{1-40}; D Aβ_{1-42}, HCHWA-D Aβ_{1-42}.

number of viable cells in culture, APP expression further increased between day 4 and 6 of the 6-day HCHWA-D Aβ_{1-40} treatment period.

In parallel experiments, HBPs and HLSMCs were treated with HCHWA-D Aβ_{1-40} 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 Aβ_{1-40} 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 Aβ_{1-40} 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 Aβ_{1-40}; 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 Aβ_{1-40} 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 Aβ_{1-40} 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 Aβ_{1-40}, but not the longer isoform HCHWA-D Aβ_{1-42}, 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 Aβ_{1-42} but not with Aβ_{1-40}. These results are in agreement with several studies describing the effects of synthetic Aβ peptides on cultured HLSMCs. Both HCHWA-D Aβ_{1-40} and Aβ_{1-42}, 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-

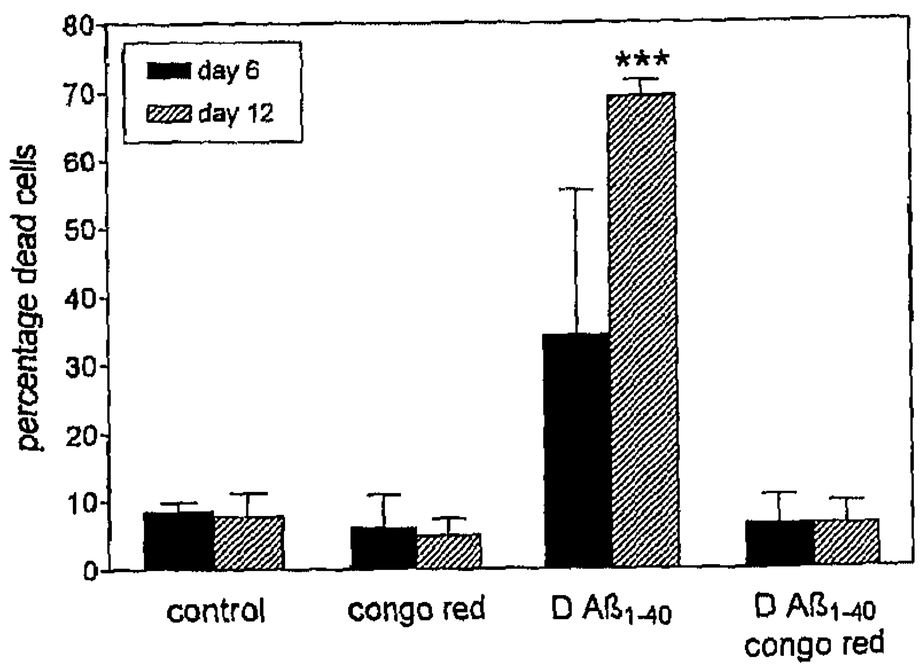

FIG. 4. Effects of exposure to HCHWA-D Aβ_{1-40} (D Aβ_{1-40}) and Congo red on the viability of HBP cultures after 6 or 12 days of incubation. Congo red entirely inhibited the degenerative effects of D Aβ_{1-40}. Data are mean ± SD (bars) values of three or more counts in a representative experiment. Statistical analysis was performed as described in the legend to Fig. 2.

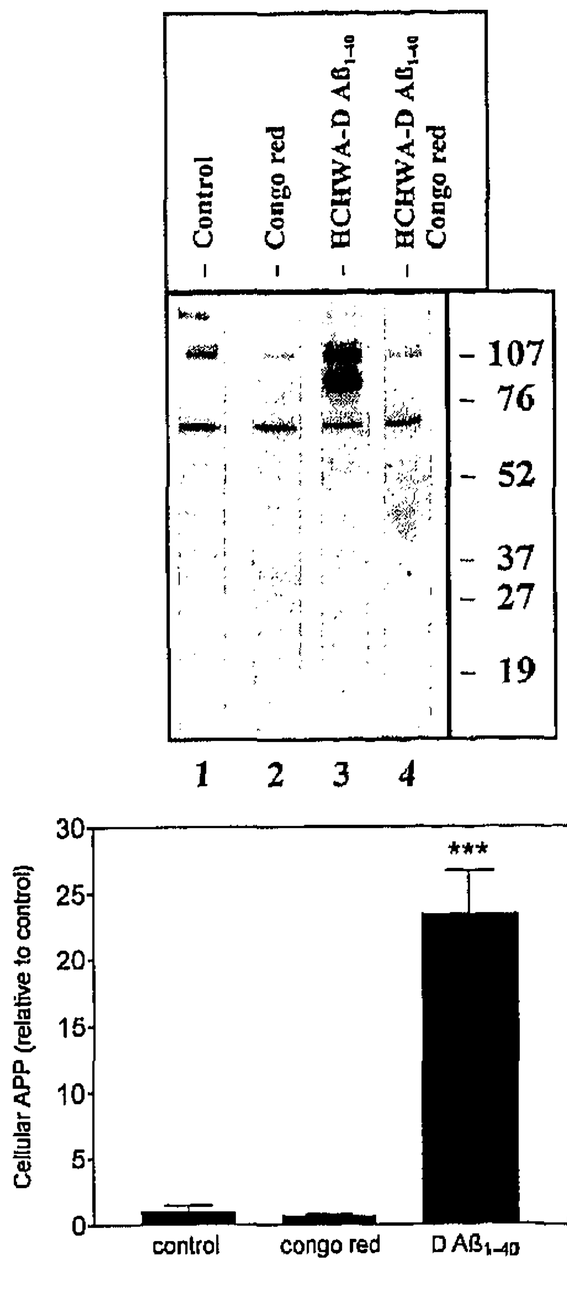

FIG. 5. Effects of HCHWA-D $A\beta_{1-40}$ and Congo red on cellular APP levels in HBP cultures. **Top:** Immunoblot analysis of cellular APP levels in cultured HBP incubated with control medium (lane 1), Congo red (25 μM; lane 2), HCHWA-D $A\beta_{1-40}$ (25 μM; lane 3), or HCHWA-D $A\beta_{1-40}$ and 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 $A\beta_{1-40}$ (D $A\beta_{1-40}$) 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.

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

However, quantitative differences could be observed in the effects of $A\beta$ on cultured HBPs or HLSMCs. In cultured HBPs, in contrast to cultured HLSMCs, treatment with HCHWA-D $A\beta_{1-40}$ 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 $A\beta_{1-40}$ 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 neighboring parenchyma (Scholz, 1938; Morel and Wildi, 1952). As cultured HBPs are more sensitive to $A\beta$ treatment and as capillaries only contain one layer of pericytes, in contrast to arteries, which contain multiple layers of SMCs, $A\beta$-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 $A\beta$-induced cellular degeneration and the induction of APP production. The increase in cellular APP production is directly related to the presence of active $A\beta$ isoforms, such as HCHWA-D $A\beta_{1-40}$, 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 $A\beta$ (Davis-Salinas et al., 1995) and of C-terminal fragments of APP (authors' unpublished

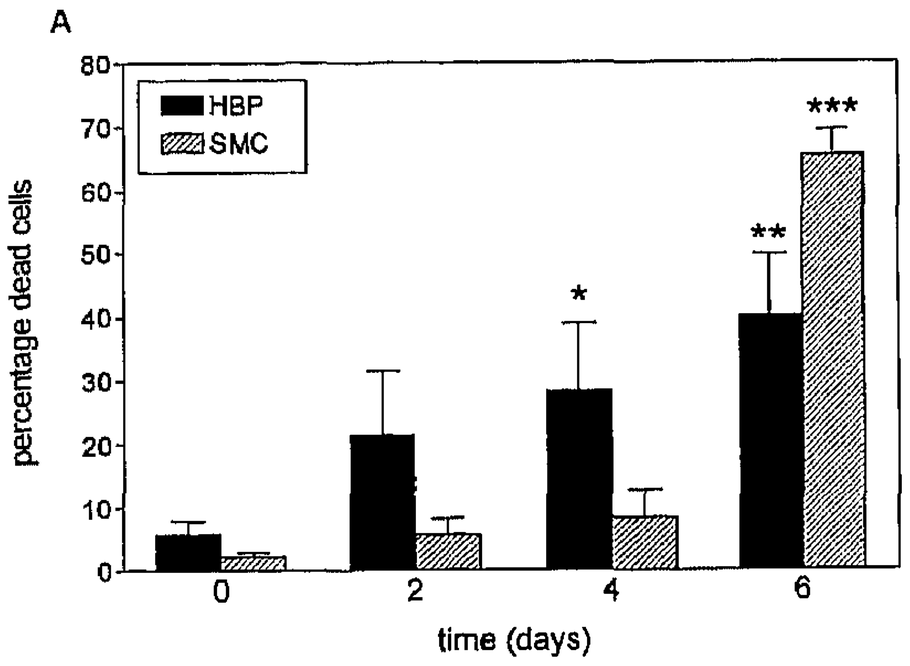

FIG. 6. Effect of short-term incubation with HCHWA-D $A\beta_{1-40}$ (0, 2, 4, or 6 days) on (**A**) viability and (**B**) cellular APP levels of HBP and SMC cultures. Data are mean ± SD (bars) values of a representative experiment. Statistical analysis was performed using Student's *t* test. The level of significance of the difference from control values is indicated as follows: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$. $p \geq 0.05$ 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.

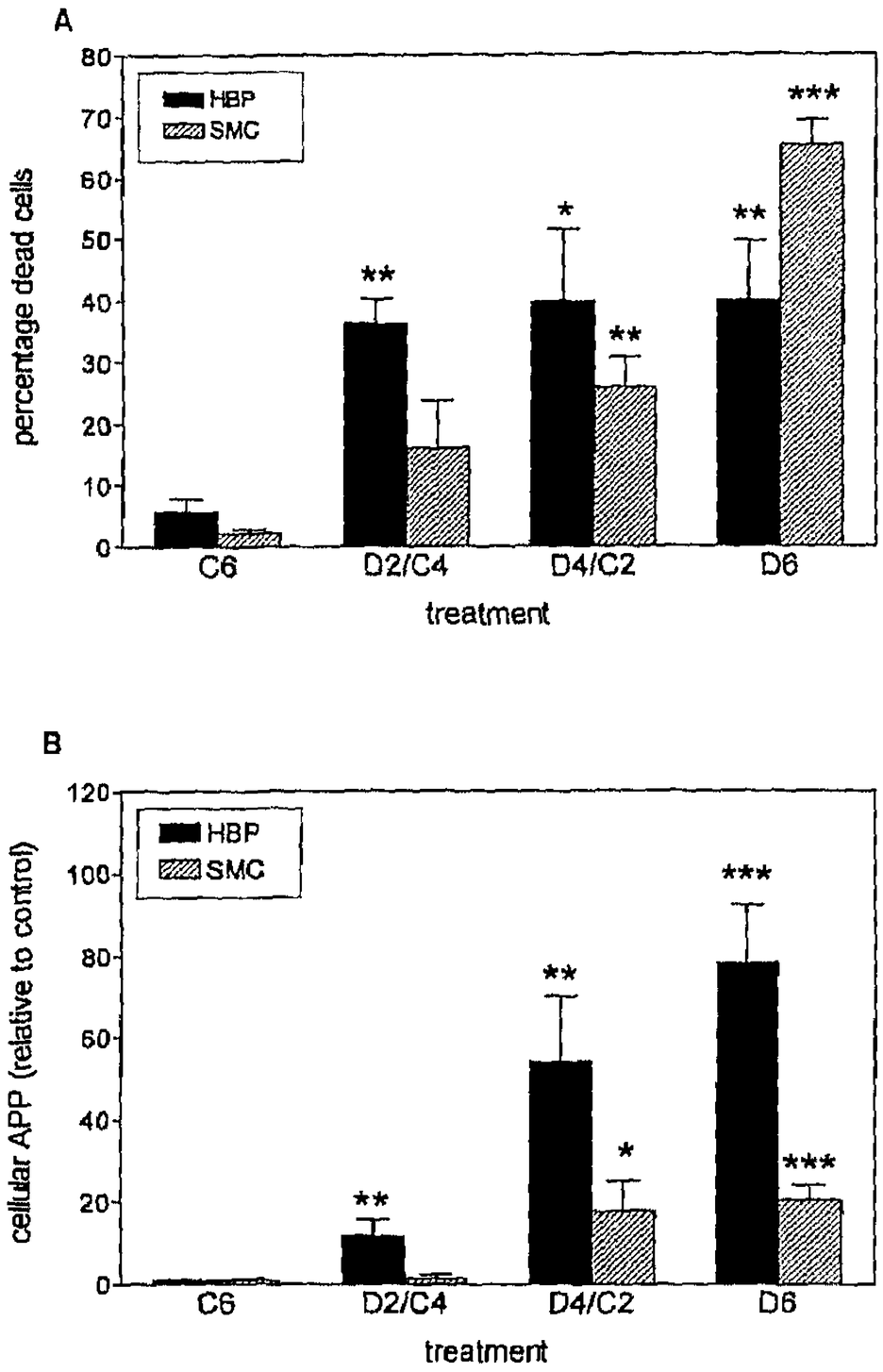

FIG. 7. Effect of short-term incubation with HCHWA-D $A\beta_{1-40}$ (0, 2, 4, or 6 days), followed by a recovery period in control medium for 6, 4, 2, or 0 days, respectively, on the (**A**) viability and (**B**) cellular APP levels of HBP and SMC cultures. Data are mean ± SD (bars) values of a representative experiment. Statistical analysis was performed as described in the legend to Fig. 6. C, incubation with control medium for the indicated number of days; D, similar incubation with medium containing HCHWA-D $A\beta_{1-40}$.

data), which may thus contribute to the process of degeneration via an amplification loop. In contrast, however, induction of cell death seemed to have different dynamics. After a short-term treatment with HCHWA-D $A\beta_{1-40}$ and subsequent removal of the 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 $A\beta$-induced cell death are not clarified yet, but the experiments with Congo red point to a mechanism in which $A\beta$ assembly may be the crucial factor similar to what has been described for the neurotoxic effects of $A\beta$ (Lorenzo and Yankner, 1994). Congo red inhibits neurotoxicity of $A\beta$ 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 $A\beta_{1-40}$, suggesting that $A\beta$ assembly is a common mechanism in the destructive effects of $A\beta$ on various cell types. If assembly of $A\beta$ is necessary for its toxicity, the aggregation rate of each $A\beta$ isoform may be decisive for its effect. Wild-type $A\beta_{1-42}$ fibrillizes faster than $A\beta_{1-40}$ (Jarrett et al., 1993), and in accordance with this, wild-type $A\beta_{1-42}$ is toxic for cultured HBPs and SMCs, whereas $A\beta_{1-40}$ is not. Furthermore, $A\beta$ peptides containing the Dutch mutation have been described to assemble into fibrils more rapidly than wild-type $A\beta$ (Wisniewski et al., 1991; Clements et al., 1993). In line with these observations, HCHWA-D $A\beta_{1-40}$ causes degeneration of cultured HBPs and SMCs, whereas wild-type $A\beta_{1-40}$ is nonactive. In analogy, it would be anticipated that HCHWA-D $A\beta_{1-42}$ is even more toxic than HCHWA-D $A\beta_{1-40}$. However, HCHWA-D $A\beta_{1-42}$ 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 $A\beta_{1-42}$ to cultured HLSMCs (Davis-Salinas and Van Nostrand, 1995), these aggregates of HCHWA-D $A\beta_{1-42}$ are not toxic for cultured HBPs. Although the protective effect of Congo red suggests that assembly of $A\beta$ is essential for its cytotoxic effect on cultured HBPs, interaction with the cell surface during assembly seems to be crucial too, as $A\beta$ assembly in solution alone does not induce cellular degeneration.

In conclusion, $A\beta$-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 $A\beta$-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 $A\beta$-induced cytotoxicity may be the formation of $A\beta$ fibrils during interaction of $A\beta$ with target cells, although this needs further investigation. The data in this study support the important role for $A\beta$ 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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