Rifampicin inhibits the toxicity of pre-aggregated amyloid peptides by binding to peptide fibrils and preventing amyloid-cell interaction

Takami TOMIYAMA*, Hideshi KANEKO, Ken-ichiro KATAOKA, Satoshi ASANO and Noriaki ENDO

Teijin Institute for Biomedical Research, 4-3-2 Asahigaoka, Hino, Tokyo 191, Japan

Rifampicin and its analogues, *p*-benzoquinone and hydroquinone, inhibited the toxicity of preformed aggregates of human islet amyloid polypeptide, amylin, to rat pheochromocytoma PC12 cells, when preincubated with the aggregated peptide before addition to cell cultures. Immunofluorescence microscopy showed that they prevented the adhesion of amylin aggregates to the cell surface, and this effect was induced probably by their binding to peptide fibrils during preincubation. Other quinone derivatives, i.e., *p*-methoxyphenol, AA-861 and idebenone, failed to inhibit the toxicity and cell-surface adhesion of amylin aggregates. Rifampicin analogues also inhibited the toxicity of pre-aggregated amyloid β1–42 peptides, suggesting a common toxic mechanism of different amyloid peptides and their therapeutic potential for several amyloidoses.

INTRODUCTION

Human islet amyloid polypeptide, amylin, is a 37-amino-acid peptide which accumulates in the pancreas of patients with type 2 (non-insulin-dependent) diabetes mellitus (for a review, see [1]). While its function *in vivo* is not clear, amylin, along with insulin, is produced by pancreatic β-cells and secreted into the bloodstream [1]. Histopathological studies have indicated that accumulation of amylin is involved in the pathogenesis of type 2 diabetes [1]. It was recently shown that amylin is toxic to islet cells *in vitro* [2], and that the toxicity requires direct contact of peptide fibrils with the cell surface [2]. This toxic feature of amylin is similar to that of another amyloid peptide, amyloid β protein (Aβ). The Aβ, a 39–43-amino-acid peptide, is the principal component of the cerebral amyloid in Alzheimer's disease (for a review, see [3]). It was shown that Aβ is toxic to neuronal cells *in vitro* (see [4] for a review) and that the toxicity is associated with fibrillar aggregates of the peptide [5].

We previously showed that rifampicin, an antibiotic, and its analogues inhibited Aβ1–40 aggregation and its toxicity to rat pheochromocytoma PC12 cells [6,7]. The inhibitory activity of the agents correlated with their radical-scavenging ability on the hydroxyl radical, which was found to be generated in cell-free incubation of Aβ1–40 peptide [7]. Since it was shown that the Aβ aggregation process involves radical reactions [8,9], we speculated that rifampicin analogues prevent Aβ aggregation by scavenging free radicals and thereby inhibit Aβ toxicity.

Recent studies have demonstrated that different amyloid peptides, including amylin and Aβ, share a common mechanism of toxicity via intracellular reactive oxygen species (ROS) generation and/or increased Ca^{2+} accumulation [10,11]. A number of antioxidants protected cells from peptide-induced ROS accumulation and toxicity [10–12]. It is likely that rifampicin analogues inhibit Aβ toxicity not only by preventing peptide aggregation but also by scavenging Aβ-induced intracellular ROS. They may also inhibit the toxicity of other amyloid peptides, such as amylin, by the same mechanism as that for Aβ toxicity.

Thus, in the present study, we examined the effects of rifampicin and its analogues on amylin aggregation and toxicity. Here we show that rifampicin and some quinone derivatives failed to inhibit amylin fibril formation but inhibited the toxicity of aggregated peptides. This inhibitory effect was induced probably by their binding to peptide fibrils rather than by their antioxidant action. The results presented here imply the existence of a mechanism, other than scavenging of free radicals, by which rifampicin and its analogues inhibit amyloid toxicity.

MATERIALS AND METHODS

Aggregation studies

Rifampicin (Sigma, St. Louis, MO, U.S.A.) and other agents (Wako Pure Chemical Industries, Osaka, Japan) were purchased, but idebenone was prepared in our laboratory by ethyl acetate extraction from an aqueous solution of Avan tablets (Takeda Chemical Industries, Osaka, Japan), a cerebral anti-ischaemic agent, and its structure was confirmed by ^{1}H NMR. All test agents were dissolved in DMSO at concentrations of 20 mM and 2 mM. Human amylin (Peptide Institute, Osaka, Japan) was initially solubilized in DMSO at a concentration of 200 μM by sonication for 10 min. The solution was diluted 5-fold with double-deionized water and then mixed with an equal volume of $2\times$PBS to give a final peptide concentration of 20 μM. The peptide solution was dispensed into Eppendorf tubes (100 μl/tube) and mixed with 1 % of each test agent in triplicate, resulting in a peptide/agent molar ratio of 1:10 or 1:1. Control peptide solutions mixed with 1 μl of DMSO were also prepared. After incubation at 37 °C for 7 days, the peptide solutions were examined for peptide fibrils by electron microscopy using an H-7100 electron microscope (Hitachi, Tokyo, Japan) at 75 kV, as described previously [6].

Abbreviations used: ROS, reactive oxygen species; Aβ, amyloid β protein; ThT, thioflavin T; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 10 % FCS/5 % HS/DMEM, Dulbecco's modified Eagle medium supplemented with 10 % fetal-calf serum and 5 % horse serum; FITC, fluorescein isothiocyanate; RAGE, receptor for advanced glycation end products.

* To whom correspondence should be addressed.

To evaluate the effects of the agents on pre-aggregated peptides, amylin and Aβ1–42 peptide (Bachem Feinchemikalien, Bubendorf, Switzerland) were allowed to aggregate by incubation at 37 °C for 3 days and 7 days respectively at a peptide concentration of 20 μM in 10 % DMSO/PBS. Peptide aggregation was measured by the thioflavin T (ThT) fluorescence assay, as described previously [6]. The aggregated peptide solutions were dispensed into tubes and mixed with each test agent in quadruplicate, as described above. After another 7 day incubation, peptide fibrils were observed under the electron microscope.

Toxicity assay

For toxicity assay, the peptide solutions incubated with each test agent were centrifuged at 100000 ***g*** for 20 min to remove soluble peptides and unbound agents. The pellets of aggregated peptides were resuspended in PBS in a volume equal to that of the supernatant. The toxicity of the peptides was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction using rat phaeochromocytoma PC12 cells and Syrian-hamster β-cell line HIT-T15 cells. Although the MTT assay is a measure of mitochondrial function and not cell death *per se*, inhibition of MTT reduction was shown to be an early indicator of the mechanism of Aβ-mediated cell death [13]. Human amylin, as well as Aβ, is toxic to clonal and primary neurons probably by the same mechanism as that of Aβ [10,11,14] and the PC12 cell, which can be differentiated into a neuron with nerve growth factor β, was shown to be more sensitive to Aβ toxicity than primary neurons, whether undifferentiated or differentiated [13]. Thus this cell line was expected to be sensitive to amylin toxicity as well as Aβ toxicity and, therefore, employed in the toxicity assay in addition to HIT-T15 cell. PC12 cells were obtained from the Riken Cell Bank (Ibaragi, Japan) and cultured in Dulbecco's modified Eagle medium supplemented with 10 % fetal-calf serum and 5 % horse serum (10 % FCS/5 % HS/DMEM). The cells were seeded into poly-L-lysine-coated 96-well culture plates at a density of 10000 cells/200 μl per well. HIT-T15 cells were obtained from the American Type Culture Collection, cultured in 10 % FCS/DMEM, and seeded into 96-well culture plates at the same density as the PC12 cells. On the day after cell seeding, 10 μl of peptide sample was added to the cell culture, giving a maximal peptide concentration of 1 μM in culture medium. In the pretreatment experiments, the cells were initially incubated with each test agent at 10 μM or 1 μM for 1 h, and then exposed to the aggregated peptides at a peptide concentration of 1 μM. After 1 day's incubation, the MTT assay was performed essentially as described previously [13]. The supernatants of the centrifuged peptides were also assayed and found to be non-toxic (results not shown).

Immunofluorescence microscopy

PC12 cells were seeded into the wells of poly-L-lysine-coated four-well Lab-Tek chamber slides (Nunc, Naperville, IL, U.S.A.) at a density of 50000 cells/500 μl per well. On the next day, the pre-aggregated amylin solutions incubated with each test agent were added to the cell culture at a peptide concentration of 1 μM. After a 1-day incubation, the cells were washed three times with PBS containing 1.8 mM Ca^{2+} and 0.8 mM Mg^{2+} [PBS (+)] and then stained with an antibody to amylin. Rabbit antiserum to rat amylin (Peptide Institute), which was shown to cross-react with human amylin, was diluted in PBS (+) containing 1 % BSA [1 % BSA/PBS (+)] and allowed to react with the cells at room temperature for 20 min. The cells were washed twice with PBS (+) and incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG antibody (Tago, Burlingame, CA, U.S.A.) in 1 % BSA/PBS (+) for 20 min. After washing again, the cells were mounted with PBS (+) and viewed under a BH-2 fluorescence microscope (Olympus).

RESULTS

Initially we determined the time courses of amylin and Aβ1–42 aggregation by ThT fluorescence assay (Figure 1). The effects of the test agents on amylin fibril formation and toxicity were examined by electron microscopy and the MTT assay using PC12 cells and HIT-T15 cells. When freshly prepared amylin was preincubated with *p*-benzoquinone for 7 days before addition to cell cultures, its fibril formation and toxicity were markedly inhibited (Figures 2, 3A and 3B). On the other hand, rifampicin and hydroquinone failed to inhibit amylin fibril formation (Figure 2), but they nevertheless attenuated its toxicity (Figures 3A and 3B). Although these two agents appeared to enlarge the width of the peptide fibrils compared with the DMSO control, it is not clear whether this difference is of significance. Previously, we showed that rifampicin and its analogues, including *p*-benzoquinone and hydroquinone, inhibited Aβ1–40 toxicity by preventing peptide aggregation, and their inhibitory activity was associated with their radical-scavenging function. The above results, however, indicate that at least rifampicin and hydroquinone inhibit amylin toxicity by a mechanism other than inhibition of peptide aggregation.

To confirm the above results, aggregated amylin was prepared by incubation of the peptide for 3 days. The aggregated amylin was then incubated with test agents for another 7 days and added to cell cultures. PC12 cells were more sensitive to amylin toxicity than HIT-T15 cells (see Figures 3A and 3B); therefore we used only PC12 cells in subsequent studies. Rifampicin, *p*-benzoquinone and hydroquinone inhibited the toxicity of the pre-aggregated amylin at a peptide/agent molar ratio of 1:10 (Figure 3C). To investigate the mechanism, other quinone derivatives were also tested. *p*-Methoxyphenol, which was previously shown not to quench the hydroxyl radical or inhibit Aβ1–40 aggregation or toxicity [7], did not show any inhibition of the toxicity of pre-aggregated amylin at the same molar concentration as rifampicin (Figure 3C). This result appears to support the idea that the inhibitory effect of the agents is associated with their radical-scavenging ability. However, AA-861, which is a lipoxygenase inhibitor and was shown to prevent Aβ-induced intracellular ROS accumulation and toxicity [15], and idebenone, which is

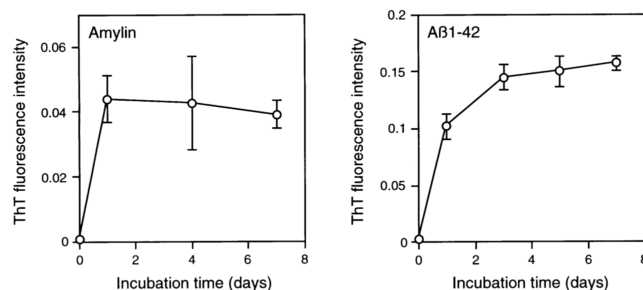

Figure 1 Time courses of amylin and Aβ1–42 aggregation

The peptides were incubated at 20 μM in 10% DMSO/PBS at 37 °C for 7 days. Peptide aggregation was monitored by the ThT fluorescence assay. Each point represents the mean ± S.D. for triplicate determinations.

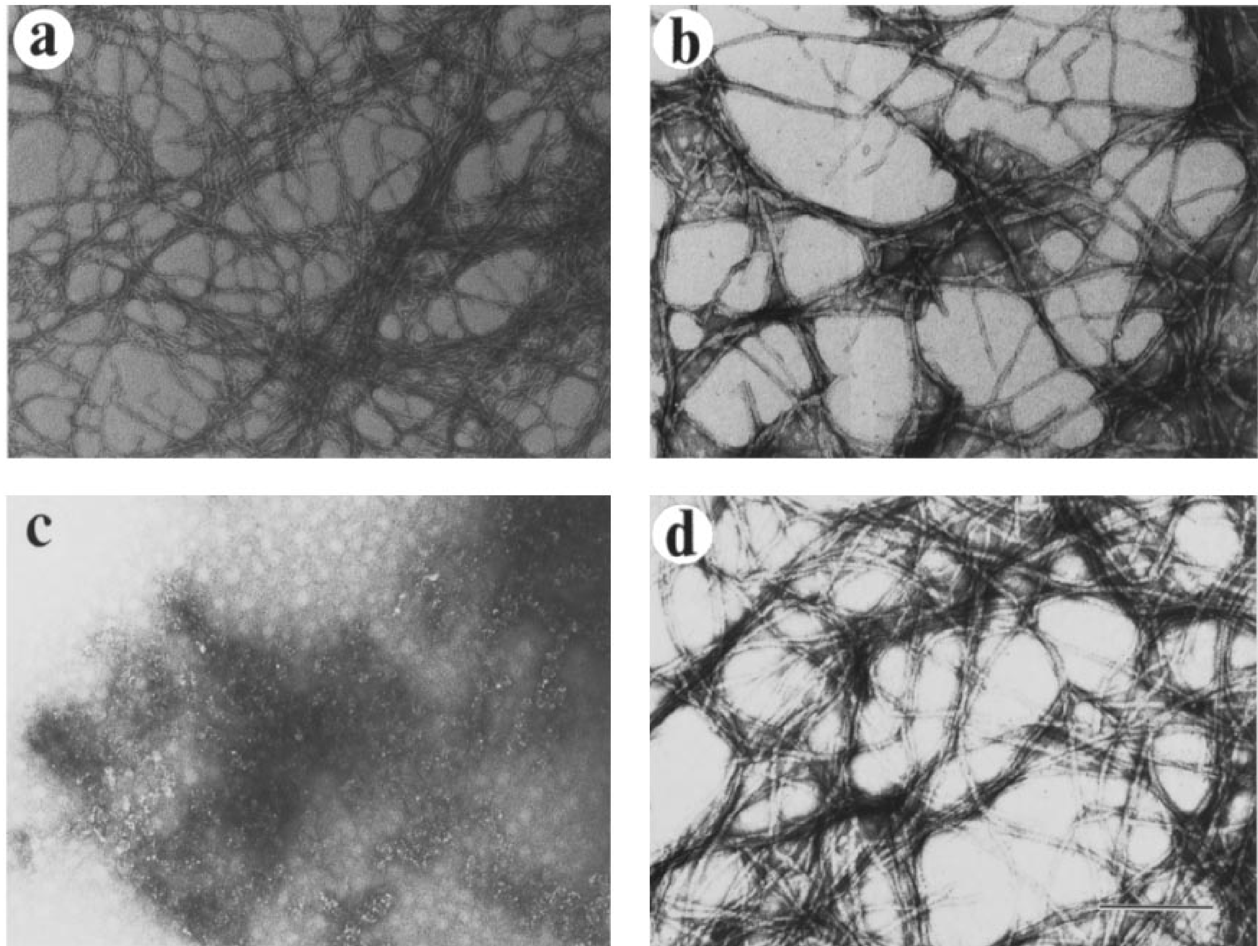

Figure 2 Effects of test agents on amylin fibril formation

Freshly-prepared soluble amylin at 20 μM in 10% DMSO/PBS was incubated at 37 °C for 7 days in the presence of each test agent at 200 μM. The control peptide solution showed apparent peptide fibrils (**a**). *p*-Benzoquinone (**c**) inhibited fibril formation, whereas rifampicin (**b**) and hydroquinone (**d**) did not. The scale bar represents 0.2 μm.

also expected to have radical-scavenging ability [16], had little effect on the toxicity of pre-aggregated amylin (Figure 3C). The tested agents did not show any trophic or toxic action to PC12 cells when added alone, except for *p*-benzoquinone, which was toxic at a final concentration of 10 μM (Figure 3D). Electron-microscopic studies showed that no significant decrease in the amount of preformed peptide fibrils was induced by rifampicin, *p*-benzoquinone or hydroquinone (results not shown). In the ThT fluorescence assay, however, the fluorescence intensity of aggregated amylin gradually decreased during incubation of the peptide with those agents (Figure 4), implying that rifampicin and its analogues bind to peptide fibrils and thereby interfere with the binding of ThT to amylin fibrils. The other three quinone derivatives, *p*-methoxyphenol, AA-861 and idebenone, on the other hand, showed little or only a moderate reduction in the ThT fluorescence. These results indicate that the inhibitory activity of the agents for pre-aggregated amylin toxicity correlates with their ability to bind to peptide fibrils but not with their radical-scavenging ability.

Then we speculated that rifampicin, *p*-benzoquinone and hydroquinone might inhibit amylin toxicity by preventing the direct contact of peptide aggregates with the cell surface. To test this hypothesis, PC12 cells were exposed for 1 day to the aggregated amylin preincubated with each test agent and then stained with an antibody to amylin. In the control cell culture, amylin aggregates were found to adhere to the cells even after several washes with PBS (Figure 5). When aggregated amylin was preincubated with rifampicin, *p*-benzoquinone or hydroquinone, it was easily washed out of the cell culture, whereas aggregates preincubated with *p*-methoxyphenol, AA-861 or idebenone remained adhered to the cells after the wash (Figure 5). These observations support our speculation that rifampicin and its analogues inhibit amylin toxicity by preventing amyloid–cell interaction. However, perhaps they inhibit the adhesion of amylin aggregates by blocking amyloid receptors on the cell surface. Thus we examined the effects of pretreatment of the cells with agents on the toxicity and cell-surface adhesion of aggregated amylin. PC12 cells were initially incubated with each test agent for 1 h and then exposed to aggregated amylin. None of the tested agents inhibited amylin toxicity, even at a peptide/agent molar ratio of 1:10 (results not shown). They also failed to inhibit the cell-surface adhesion of amylin aggregates (Figure 5). Consequently, we propose that rifampicin, *p*-benzoquinone and hydroquinone inhibit the toxicity of pre-aggregated amylin by

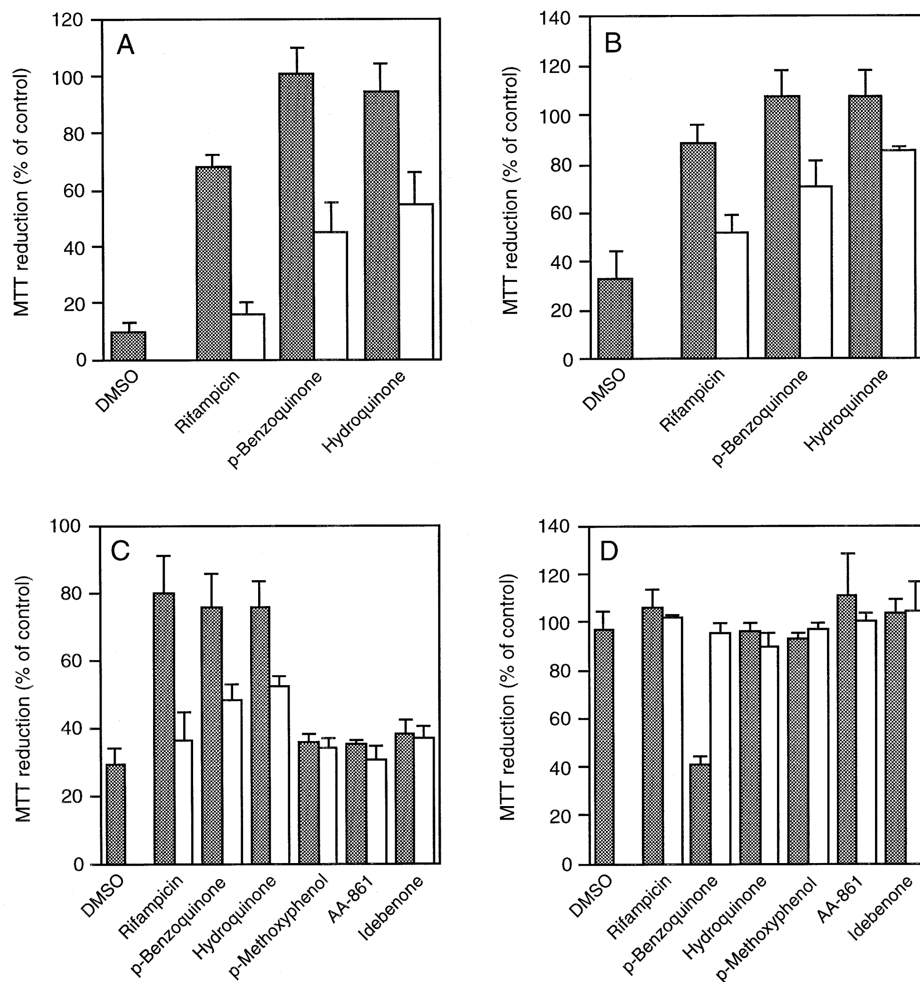

Figure 3 Effects of test agents on amylin toxicity

The toxicity of the peptides and the agents was assessed by MTT reduction with rat pheochromocytoma PC12 cells (**A**, **C** and **D**) and Syrian-hamster β-cell line HIT-T15 cells (**B**). (**A** and **B**) Amylin at 20 μM was incubated at 37 °C for 7 days with each test agent at a peptide/agent molar ratio of 1:10 (shaded column) or 1:1 (open column). The peptide solutions were centrifuged at 100000 ***g*** for 20 min to remove soluble peptides and unbound agents. The pellets of aggregated peptides were resuspended in PBS in a volume equal to that of the supernatant. Then 10 μl of peptide sample was added to the cell culture (200 μl/well), giving a maximal peptide concentration of 1 μM in the culture medium. After 1 day's incubation, the MTT assay was performed. (**C**) Amylin was allowed to aggregate by incubation at 37 °C for 3 days at a peptide concentration of 20 μM in 10% DMSO/PBS. The aggregated peptide was then incubated at 37 °C for another 7 days with each test agent at a peptide/agent molar ratio of 1:10 (shaded column) or 1:1 (open column). The peptide solutions were centrifuged, and the pellets were resuspended in PBS and added to PC12 cells, as described above. (**D**) 10 μl of each test agent diluted in PBS was added to the cell culture at a final concentration of 10 μM (shaded column) or 1 μM (open column). Values represent the means $\pm$ S.D. for triplicate (**A** and **B**) or quadruplicate (**C** and **D**) determinations.

interacting directly with the peptide rather than by blocking its possible receptors or by affecting intracellular events, such as ROS accumulation and MTT reduction.

It is to be expected that rifampicin and its analogues may also inhibit the toxicity of pre-aggregated Aβ. Thus the effects of the agents were examined on the toxicity of aggregated Aβ1–42 peptide, which was prepared by incubation for 7 days (see Figure 1). After 7 days preincubation with the aggregated peptide, rifampicin, *p*-benzoquinone and hydroquinone inhibited Aβ1–42 toxicity, whereas *p*-methoxyphenol, AA-861 and idebenone had little effect (Figure 6). Electron-microscopic studies again showed that rifampicin analogues induced no significant decrease in the amount of peptide fibrils (results not shown). Moreover, the ThT fluorescence of aggregated Aβ1–42 decreased gradually in the presence of those agents (results not shown). These results suggest that rifampicin, *p*-benzoquinone and hydroquinone inhibit the toxicity of pre-aggregated Aβ by the same mechanism as that for pre-aggregated amylin.

DISCUSSION

The toxicity of Aβ has been implicated in the neuronal degeneration of Alzheimer's disease. Similarly, a relationship of amylin to concurrent islet-cell dysfunction has been suggested in the pathogenesis of type 2 diabetes. *In vitro* studies have shown that the toxicity of these peptides requires fibril formation of the peptides [2,5]. The amyloid-binding dye Congo Red was shown to inhibit the cytotoxicity of those peptides by preventing their fibril formation or by binding to the preformed fibrils [5]. Those observations suggest that inhibition of peptide aggregation or prevention of amyloid–cell interaction leads to protection of cells from amyloid toxicity. We previously demonstrated that

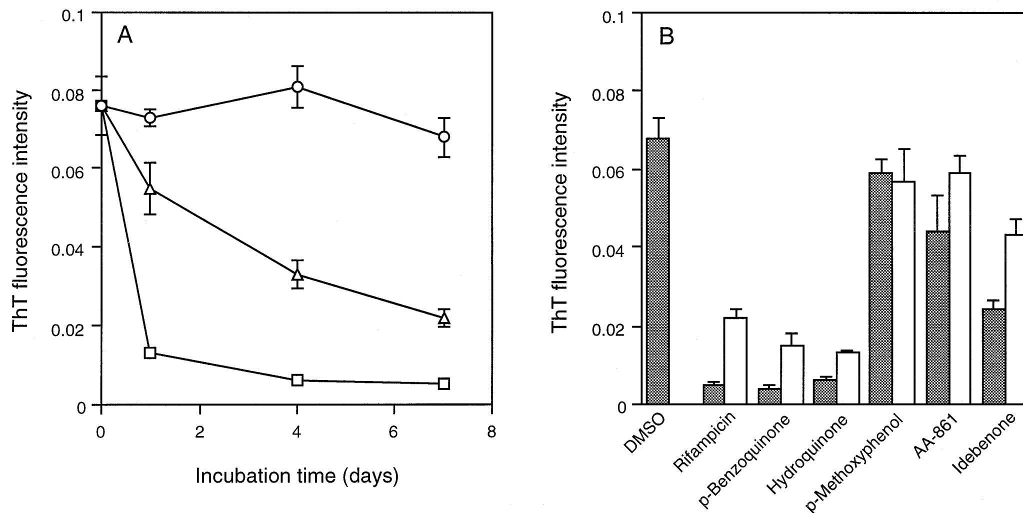

Figure 4 Effects of test agents on the ThT fluorescence of pre-aggregated amylin

Pre-aggregated amylin at 20 μM was incubated at 37 °C for 7 days in the presence of each test agent. (**A**) the ThT fluorescence intensity of aggregated amylin decreased gradually during incubation of the peptide with 200 μM (□) or 20 μM (△) rifampicin, but not with DMSO (○). *p*-Benzoquinone and hydroquinone also attenuated the fluorescence in a time- and concentration-dependent manner, but their effects at 20 μM were more potent than that of rifampicin (results not shown). (**B**) Compared with those agents, three other quinone derivatives showed little or only a moderate decrease in the fluorescence at 200 μM (shaded column) or 20 μM (open column), even after the 7-day incubation. Values represent the means ± S.D. for quadruplicate determinations.

rifampicin and its analogues, including *p*-benzoquinone and hydroquinone, inhibited Aβ toxicity by preventing aggregation of this peptide [6,7]. And here we have shown that rifampicin, *p*-benzoquinone and hydroquinone also inhibit the cell-surface adhesion and toxicity of amylin aggregates, probably by binding to peptide fibrils. The anti-amyloid profiles of the rifampicin analogues resemble those of Congo Red as reported, while their molecular structures appear not to be similar.

The rifampicin analogues inhibited the binding of ThT, another amyloid-binding dye, to peptide fibrils, suggesting that they bind to amyloid-specific structures. Interestingly, ThT itself did not inhibit the cell-surface adhesion of aggregated amylin (results not shown). Recently, two candidates for amyloid receptor on the cell surface were identified: the receptor for advanced glycation end products (RAGE) [17] and the class A scavenger receptor [18]. RAGE was shown to mediate the interaction of Aβ with endothelial cells and neurons, including PC12 cells, to cause cellular oxidant stress, and with microglia to induce cellular activation [17]. On the other hand, the class A scavenger receptor is expressed on mononuclear phagocytes, including microglia, and was shown to mediate the adhesion of microglia to Aβ fibrils, resulting in ROS generation [18]. Since it was suggested that unidentified structural determinants on Aβ which are distinct from advanced glycation end products are critical to Aβ–RAGE interaction [17], the binding of rifampicin analogues, but not ThT, to peptide fibrils may inhibit this interaction by altering their conformation or hydrophobicity. Two other antioxidant quinone derivatives, AA-861 and idebenone, had little or only a moderate effect on the ThT fluorescence or cell-surface adhesion of aggregated amylin. Thus the radical-scavenging function of the agents is not sufficient, if necessary, for their binding to peptide fibrils. It is unlikely that rifampicin analogues inhibit the adhesion of amylin aggregates by blocking amyloid receptors on the cell surface, because pretreatment of the cells with those agents had no effect on amylin toxicity. These results also rule out the possibility that the inhibition of amylin toxicity by the agents is an artifact caused by their interaction with the assay system, thereby inducing enhanced MTT reduction.

In addition to amylin and Aβ, many amyloid peptides associated with human amyloidoses have been identified. Although those peptides show different amino acid sequences, their aggregated forms are characterized by an antiparallel β-sheet conformation [19]. Recent studies have shown that different amyloid peptides, including amylin and Aβ, share a common mechanism of toxicity via intracellular ROS and/or Ca^{2+} accumulation [10,11]. A number of antioxidants protected cells from peptide-induced ROS accumulation and toxicity [10–12], and the mechanism of the inhibition has been speculated to be detoxifying of intracellular ROS. Taken together with previous observations [2,4,5], those intracellular events leading to cell death are probably induced mainly by aggregated forms of the peptides. In fact, it was shown that Aβ-induced Ca^{2+} accumulation is associated with peptide aggregation on the cell surface [20]. If this were the case, rifampicin analogues with radical-scavenging ability as well as other antioxidants, such as AA-861 [15], could also inhibit the toxicity of pre-aggregated amyloid peptides by counteracting intracellular ROS. In the present study, however, they failed to inhibit the toxicity of pre-aggregated amylin when added to cell cultures prior to the peptide. These results indicate that the antioxidants reported to inhibit amyloid toxicity exhibit the effect by mechanisms other than detoxifying of intracellular ROS. They may inhibit the aggregation of soluble peptides in the cell-culture medium by scavenging free radicals, which results in prevention of intracellular ROS generation. Alternatively, the toxic mechanism of peptide aggregates preformed under non-biological conditions may not involve ROS generation, differing from that of the gradually aggregating peptides associated with the cell surface in the culture medium.

In summary, we have shown that rifampicin and the two structurally related agents inhibit the toxicity of pre-aggregated amylin and pre-aggregated Aβ1–42, probably by binding to peptide fibrils and subsequently preventing adhesion of the pep-

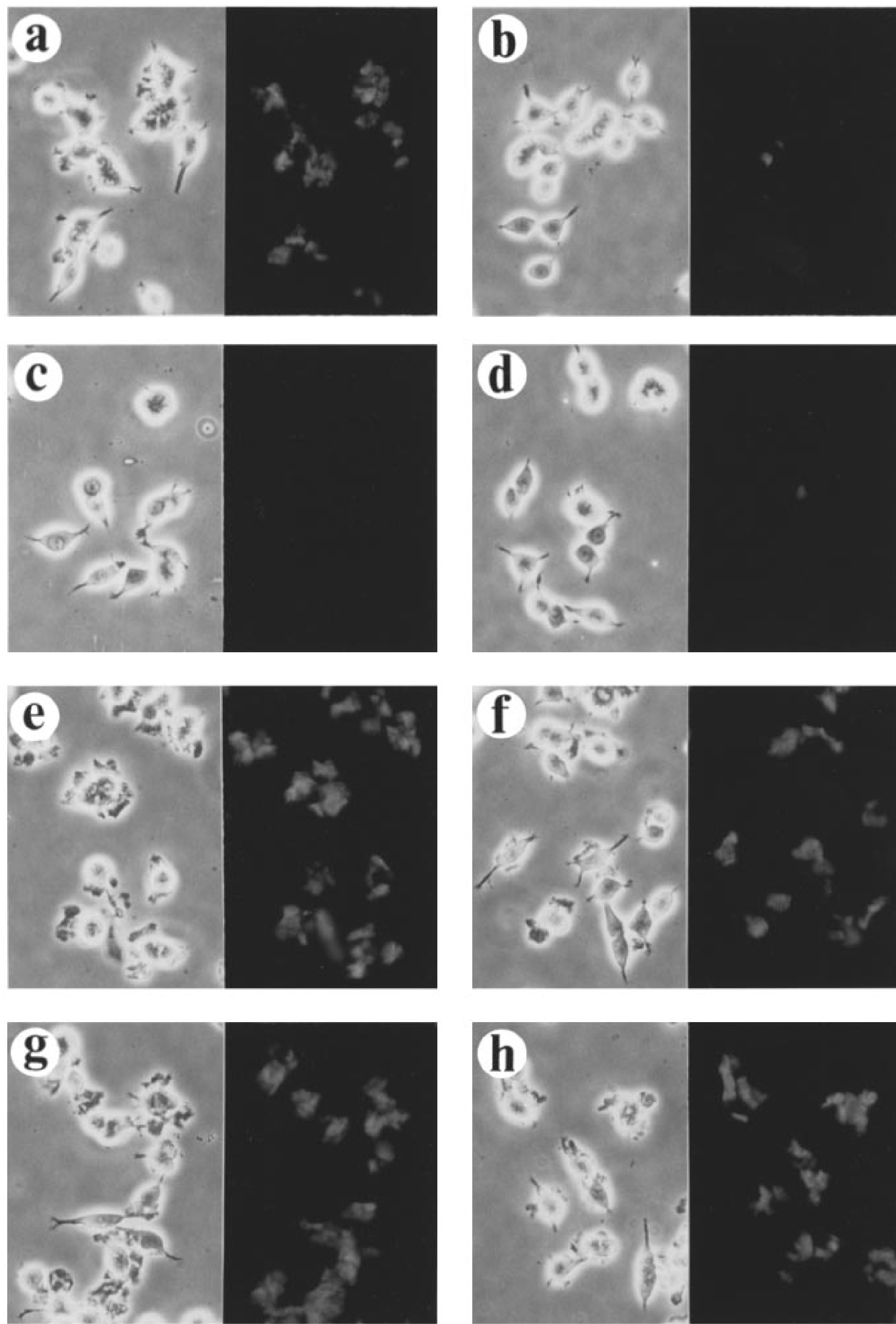

Figure 5 Effects of test agents on cell-surface adhesion of amylin aggregates

Pre-aggregated amylin at 20 μM was incubated with each test agent at 200 μM at 37 °C for 7 days. The peptide solutions were added to PC12 cells at a peptide concentration of 1 μM. After a 1 day incubation, the cells were washed three times with PBS and then stained with an antibody to amylin, followed by an FITC-conjugated second antibody. In the control, amylin aggregates preincubated with DMSO (**a**) were found to adhere to the cells even after several washes with PBS. Rifampicin (**b**), *p*-benzoquinone (**c**) and hydroquinone (**d**) inhibited the cell-surface adhesion of amylin aggregates, whereas *p*-methoxyphenol (**e**), AA-861 (**f**) and idebenone (**g**) did not. To examine the effects of pretreatment with the agents, the cells were initially incubated with each test agent at 10 μM for 1 h and then exposed to 1 μM aggregated amylin. Neither rifampicin (**h**) nor the other tested agents inhibited the binding of amylin aggregates to the cell surface.

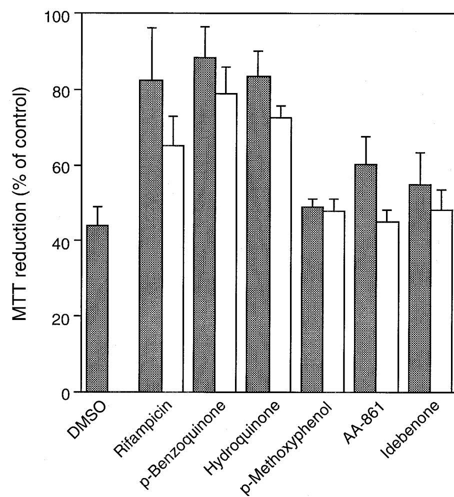

Figure 6 Effects of test agents on pre-aggregated Aβ1–42 toxicity

Initially Aβ1-42 was allowed to aggregate by incubation at 37 °C for 7 days at a peptide concentration of 20 μM in 10% DMSO/PBS. The aggregated peptide was then incubated at 37 °C for another 7 days with each test agent at a peptide/agent molar ratio of 1:10 (shaded column) or 1:1 (open column). The peptide solutions were centrifuged, and the pellets were resuspended in PBS and added to PC12 cells, as described for Figure 3. After a 1 day incubation the MTT assay was performed. Values represent the means ± S.D. for quadruplicate determinations.

tide aggregates to the cell surface. Taken together with our previous findings, it was suggested that those agents inhibit amyloid toxicity by two mechanisms, i.e., radical-scavenging, which inhibits peptide aggregation, and amyloid-binding, which inhibits amyloid–cell interaction. Our data presented here provide useful information for investigating the mechanisms of amyloid toxicity and for developing new compounds for the treatment of type 2 diabetes, Alzheimer's disease and several other amyloidoses.

29 August 1996/30 September 1996; accepted 7 November 1996

REFERENCES

1 Johnson, K. H., O'Brien, T. D., Betsholtz, C. and Westermark, P. (1992) Lab. Invest. **66**, 522–535
2 Lorenzo, A., Razzaboni, B., Weir, G. C. and Yankner, B. A. (1994) Nature (London) **368**, 756–760
3 Selkoe, D. J. (1994) Annu. Rev. Cell Biol. **10**, 373–403
4 Iversen, L. L., Mortishire-Smith, R. J., Pollack, S. J. and Shearman, M. S. (1995) Biochem. J. **311**, 1–16
5 Lorenzo, A. and Yankner, B. A. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 12243–12247
6 Tomiyama, T., Asano, S., Suwa, Y., Morita, T., Kataoka, K., Mori, H. and Endo, N. (1994) Biochem. Biophys. Res. Commun. **204**, 76–83
7 Tomiyama, T., Shoji, A., Kataoka, K., Suwa, Y., Asano, S., Kaneko, H. and Endo, N. (1996) J. Biol. Chem. **271**, 6839–6844
8 Dyrks, T., Dyrks, E., Hartmann, T., Masters, C. and Beyreuther, K. (1992) J. Biol. Chem. **267**, 18210–18217
9 Hensley, K., Carney, J. M., Mattson, M. P., Aksenova, M., Harris, M., Wu, J. F., Floyd, R. A. and Butterfield, D. A. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 3270–3274
10 Schubert, D., Behl, C., Lesley, R., Brack, A., Dargusch, R., Sagara, Y. and Kimura, H. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 1989–1993
11 Mattson, M. P. and Goodman, Y. (1995) Brain Res. **676**, 219–224
12 Behl, C., Davis, J. B., Lesley, R. and Schubert, D. (1994) Cell **77**, 817–827
13 Shearman, M. S., Ragan, C. I. and Iversen, L. L. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 1470–1474
14 May, P. C., Boggs, L. N. and Fuson, K. S. (1993) J. Neurochem. **61**, 2330–2333
15 Goodman, Y., Steiner, M. R., Steiner, S. M. and Mattson, M. P. (1994) Brain Res. **654**, 171–176
16 Oka, A., Belliveau, M. J., Rosenberg, P. A. and Volpe, J. J. (1993) J. Neurosci. **13**, 1441–1453
17 Yan, S. D., Chen, X., Fu, J., Chen, M., Zhu, H., Roher, A., Slattery, T., Zhao, L., Nagashima, M., Morser, J. et al. (1996) Nature (London) **382**, 685–691
18 Khoury, J. E., Hickman, S. E., Thomas, C. A., Cao, L., Silverstein, S. C. and Loike, J. D. (1996) Nature (London) **382**, 716–719
19 Sipe, J. D. (1992) Annu. Rev. Biochem. **61**, 947–975
20 Mattson, M. P., Barger, S. W., Cheng, B., Lieberburg, I., Smith-Swintosky, V. L. and Rydel, R. E. (1993) Trends Neurosci. **16**, 409–414