Extracellular chaperones modulate the effects of Alzheimer's patient cerebrospinal fluid on A β ₁₋₄₂ toxicity and uptake

Justin J. Yerbury · Mark R. Wilson

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Abstract Alzheimer's disease is characterised by the inappropriate death of brain cells and accumulation of the A β peptide in the brain. Thus, it is possible that there are fundamental differences between Alzheimer's disease patients and healthy individuals in their abilities to clear A β from brain fluid and to protect neurons from A β toxicity. In the present study, we examined (1) the cytotoxicity of Alzheimer's disease cerebrospinal fluid (CSF) compared to control CSF, (2) the ability of Alzheimer's disease and control CSF to protect cells from A β toxicity and to promote cell-mediated clearance of A β and lastly (3) the effects of extracellular chaperones, normally found in CSF, on these processes. We show that the Alzheimer's disease CSF samples tested were more toxic to cultured neuroblastoma cells than normal CSF. In addition, the Alzheimer's disease CSF samples tested were less able to protect cells from A β -induced toxicity and less efficient at promoting macrophage-like cell uptake when compared to normal CSF. The addition of physiologically relevant concentrations of the extracellular chaperones, clusterin, haptoglobin and α_2 -macroglobulin into CSF protected neuroblastoma cells from A β ₁₋₄₂ toxicity and promoted A β ₁₋₄₂ uptake in macrophage-like cells. These results suggest that extracellular chaperones are an impor-

tant element of a system of extracellular protein folding quality control that protects against A β toxicity and accumulation.

Keywords A β · Alzheimer's disease · CSF · Extracellular chaperones

Abbreviations

ECs	Extracellular chaperones
BSA	Bovine serum albumin
LRP	Low density lipoprotein receptor related protein
α_2 m	α_2 -macroglobulin
Hp	Haptoglobin
A β	Amyloid beta peptide
QC	Quality control
CSF	Cerebrospinal fluid
ALS	Amyotrophic lateral sclerosis

Introduction

A β is the major constituent in extracellular plaques characteristic of Alzheimer's disease and its increased expression is thought to be the triggering event leading to disease pathology (Silvestrelli et al. 2006). This is supported by the fact that, in all cases of early-onset familial Alzheimer's disease where a genetic mutation has been identified, the defective gene causes an increase in production of the more toxic processing variant A β ₁₋₄₂ (Mattson 2004; Iwata et al. 2005). The actual mechanism by which A β is toxic is unclear; there are several hypotheses. A β aggregates, such as mature fibrils (Mattson 2004) and soluble oligomers (Klein 2002), are toxic to cells *in vitro* and are thought to cause pathology in humans

J. J. Yerbury (✉) · M. R. Wilson
School of Biological Sciences, University of Wollongong,
Northfields Avenue,
Wollongong, NSW 2522, Australia
e-mail: jyerbury@uow.edu.au

J. J. Yerbury
Department of Chemistry, University of Cambridge,
Lensfield Road,
Cambridge, UK

(Gong et al. 2003). A β has also been linked to other toxic pathways involving metal ions (e.g. Fe²⁺ and Cu²⁺) and their subsequent generation of free radicals (Maynard et al. 2005). A β is also thought to trigger an inflammatory response in the brain that may underpin disease pathology (Silvestrelli et al. 2006) and be involved in cerebral vascular dysfunction (Greenberg et al. 1996). Regardless of the route of toxicity, it seems that even small increases in soluble A β _{1–42} (even as small as a 1.5-fold increase) can result in the appearance of aggressive presenile A β pathology (Iwata et al. 2005).

Alzheimer's disease is included in a group of disorders that are associated with inappropriate protein deposits, collectively known as protein deposition disorders. These include Parkinson's disease, amyotrophic lateral sclerosis and Huntington's disease which are associated with deposits of α -synuclein, Cu/Zn superoxide dismutase and huntingtin, respectively. It has been postulated that protein deposits arise when the normally efficient protein folding quality control system is overwhelmed (Muchowski and Wacker 2005; Wilson et al. 2008). However, once in the extracellular space, A β is no longer under the surveillance of the intracellular protein folding quality control (QC) system. Recently, it was proposed that an analogous extracellular protein folding QC system exists that recognises non-native proteins and promotes their removal from the extracellular fluid via receptor-mediated endocytosis (Yerbury et al. 2005a, b). It has been shown that clusterin, a ubiquitous and highly conserved secreted protein found in human blood, is a potent extracellular chaperone (Humphreys et al. 1999; Poon et al. 2000; Poon et al. 2002). More recently, both haptoglobin (Hp; previously best known for its high affinity binding to haemoglobin; Yerbury et al. 2005a,b) and α_2 -macroglobulin (α_2 m; previously best known as an inhibitor of many proteases; French et al. 2008) have been shown to possess potent chaperone actions similar to clusterin. All three of these known extracellular chaperones can be detected co-localised with "plaques" in Alzheimer's disease (Powers et al. 1981; Strauss et al. 1992; Kida et al. 1995). The reason for their presence there is unknown but it is likely that their chaperone action has been overwhelmed and that they have consequently been co-deposited with A β (Yerbury et al. 2007). In addition, clusterin, Hp and α_2 m have already been shown to inhibit amyloid fibril formation by A β *in vitro* (Hughes et al. 1998; Yerbury et al. 2007; 2009). Clusterin and α_2 m have previously been shown to complex with A β *in vitro* and facilitate its transport *via* receptor-mediated endocytosis to lysosomes for degradation (Hammad et al. 1997; Narita et al. 1997). This is consistent with *in vivo* studies in mice which show that α_2 m and clusterin increase the rate of A β removal from the brain (Shibata et al. 2000) (Bell et al. 2007).

Since it has been proposed that A β metabolism in the brain is dysfunctional in Alzheimer's disease (Iwata et al. 2005), we wondered whether there was a fundamental difference between Alzheimer's disease patients and healthy individuals in their ability to promote cellular uptake/removal of A β from CSF or to protect neurons from A β toxicity. Previous work has shown that normal human CSF inhibits the formation of A β amyloid fibrils *in vitro* while Alzheimer's disease patient CSF does not (Ono et al. 2005). In addition, previous studies have shown that CSF from patients suffering other neurodegenerative diseases such as ALS (Couratier et al. 1994) or Parkinson's (Le et al. 1999) is itself toxic to neuronal cells *in vitro*. In the present study, we examined the toxicity of Alzheimer's disease CSF compared to control CSF. In addition, we examined the ability of Alzheimer's disease and control CSF, with or without added extracellular chaperones, to protect cells from A β toxicity and to promote cell-mediated clearance of A β .

Results

Alzheimer's CSF is toxic to SY5Y cells

CSF from patients with the neurodegenerative diseases ALS and Parkinson's disease is toxic to cells *in vitro* (Couratier et al. 1994; Le et al. 1999). To determine whether Alzheimer's disease CSF is cytotoxic, SH-SY5Y neuroblastoma cells were incubated in Aim V (Invitrogen, Sydney, Australia) supplemented with a range of levels of human normal and Alzheimer's CSF for 48 h (e.g. a mixture of equal volumes of Aim V and CSF is referred to as 50% CSF). All samples were analysed individually and then the results were averaged. All CSF samples were collected and supplied by the National Neural Tissue Resource Centre (Melbourne, Australia); individual donors were confirmed as Alzheimer's or non-Alzheimer's by post mortem examination of brain sections. Alzheimer's CSF was obtained from three males aged 68, 71 and 82 (designated AD 1, 2 and 3, respectively, in Figs. 2 and 4), while non-Alzheimer's CSF was obtained from three males aged 48, 51 and 69 (designated Normal 1, 2 and 3, respectively, in Figs. 2 and 4). CSF from non-symptomatic donor tissue that contained A β plaques were excluded from the "Normal" control samples—this had the consequence that exactly age-matched controls were unavailable. Following incubation with CSF, cells were assayed for viability using calcein-AM (Lichtenfels et al. 1994). The calcein fluorescence of cells incubated with 50% (v/v) normal control CSF was not significantly different to cells incubated without added CSF (Fig. 1, Student's *t* test, *p* > 0.05), indicating that under the conditions tested normal

Fig. 1 AD CSF is toxic to SH-SY5Y cells. Loss of viability of SH-SY5Y cells, measured as calcein fluorescence and expressed as percentage of untreated control cultures. Cells were cultured for 48 h in FBS-free AIM V medium, with or without various levels of CSF. The % CSF on the x-axis corresponds to the amount of CSF present (v/v) in the media. Calcein-AM was used to measure cell viability. Calcein-AM (1 μ M) was added to cells and left to incubate for 30 min before analysing fluorescence using a FLUOstar OPTIMA plate reader with excitation and emission windows of 485 ± 10 nm and 520 ± 10 nm, respectively. Significant differences ($p < 0.05$) from the no additives control are indicated by *. The data points shown represent means of all samples from three independent experiments, with 12 samples in total, the error bars are SE

CSF was not toxic to SH-SY5Y cells. In contrast, the calcein fluorescence of cells incubated with 50% Alzheimer's CSF was significantly less. The toxicity of Alzheimer's CSF was dose dependent—the level of calcein fluorescence decreased with increasing concentrations of CSF (Fig. 1). The differences between control and Alzheimer's CSF treated wells were statistically significant at both 25% and 50% (v/v) CSF (Student's t test, $p < 0.05$). Fluorescence microscopy of calcein-AM stained cells confirmed that there were less viable cells and loss of neurite outgrowth in wells containing 25% and 50% Alzheimer's CSF compared to those incubated with control CSF (data not shown).

Addition of extracellular chaperones protects cells from Alzheimer's CSF toxicity

Immunoblotting analysis of the CSF samples used in this study showed that the levels of all three extracellular chaperones tested (α_2 m, haptoglobin and clusterin) were higher in control CSF than in Alzheimer's CSF (Fig. 2a). In addition, a BCA assay indicated that the total protein concentrations in the Alzheimer's CSF samples used in this study were on average lower than that of normal CSF (Fig. 2b). Thus, the difference in extracellular chaperone levels may be due to differences in total protein concentration between control and Alzheimer's CSF, rather than a selective depletion of extracellular chaperones in Alzheimer's CSF (Fig. 2a, b). Regardless, the differences in

extracellular chaperone levels prompted us to test whether adding exogenous extracellular chaperones into Alzheimer's CSF would protect cells from its toxicity. Compared to cells incubated with Alzheimer's CSF alone, those incubated with Alzheimer's CSF supplemented with 2 μ g/ml clusterin, 2 μ g/ml haptoglobin and 4 μ g/ml α_2 m showed significantly greater viability after 48 h (Fig. 2c; $p < 0.05$). In contrast, when the same concentrations of extracellular chaperones were added to normal CSF, this had no effect on the resulting cell viability (Fig. 2c).

Alzheimer's CSF promotes A β_{1-42} toxicity which is modulated by extracellular chaperones

A β_{1-42} was incubated under amyloid forming conditions, as previously reported (Yerbury et al. 2007), and samples removed at 2 h. When incubated with cells in the absence

Fig. 2 Extracellular chaperones protect SH-SY5Y cells from AD CSF toxicity. **a** Western blot images showing levels of α_2 m, Hp and clusterin in 5 μ l of CSF. **b** Protein concentration in CSF samples measured using BCA assay. BSA was used to create a standard curve and the assay was conducted as per the manufacturer's instructions. For (a) and (b), individual CSF samples are labelled 1–3 (see first paragraph in Results section). **c** Loss of viability of SH-SY5Y cells, measured as calcein fluorescence and expressed as percentage of untreated control cultures. Cells were cultured for 48 h in FBS-free AIM V medium, with or without individual CSF samples and/or a cocktail of 2 μ g/ml clusterin, 2 μ g/ml haptoglobin and 4 μ g/ml α_2 m. 1 μ M Calcein-AM was used to measure cell viability. Significant differences ($p < 0.05$) from the control CSF sample are indicated by *. The data points shown represent means of all samples from three independent experiments, with nine samples in total, the error bars are SE

Fig. 3 Normal CSF and extracellular chaperones protect SH-SY5Y cells from $A\beta_{1-42}$ toxicity. Loss of viability of SH-SY5Y cells, measured as calcein fluorescence and expressed as percentage of untreated control cultures, incubated for 48 h with $A\beta_{1-42}$ alone or in the presence of CSF with or without added extracellular chaperones (2 $\mu\text{g/ml}$ clusterin, 2 $\mu\text{g/ml}$ haptoglobin and 4 $\mu\text{g/ml}$ $\alpha_2\text{m}$). To induce cytotoxicity, aliquots of $A\beta_{1-42}$ reactions (taken at 2 h) were added to cells alone to give final concentrations of 1 μM . Aggregation of $A\beta_{1-42}$ (10 μM) was initiated by incubation in PBS containing 90 mM CaCl_2 , 50 mM MgCl_2 , 100 mM CuCl_2 , 600 mM glycine, pH 7.5. Significant differences from the normal CSF alone sample are indicated by *. Significant differences from the AD CSF+A β sample are indicated by #. The data points shown represent means of all samples from three independent experiments, with nine samples in total, the error bars are SE

of CSF, these prefibrillar fractions were toxic to SH-SY5Y cells in a dose-dependent manner (Yerbury et al. 2007). The possibility that CSF could protect cells from toxic $A\beta_{1-42}$ species was examined. When toxic $A\beta_{1-42}$ aggregates were added to cells incubated in 50% (v/v) normal CSF, there was no significant loss of viability (Fig. 3). In contrast, when the same dose of $A\beta_{1-42}$ aggregates were added to

cells incubated with 50% Alzheimer's CSF, a significant loss of viability resulted (Fig. 3, Alzheimer's CSF+A β versus N CSF+A β ; Student's t test, $p<0.05$). Addition of extracellular chaperones to cells incubated with A β supplemented Alzheimer's CSF provided significant protection (Student's t test, $p<0.05$). However, the protection provided by extracellular chaperones did not bring cell viability back to the level of controls (Fig. 3 Alzheimer's CSF+A β +extracellular chaperone versus N CSF+A β ; Student's t test, $p<0.05$).

Extracellular chaperones promote macrophage-mediated uptake of $A\beta_{1-42}$ from CSF

Since the metabolism of $A\beta_{1-42}$ in the brain is thought to be defective (Iwata et al. 2005), we examined the possibility that Alzheimer's CSF negatively affected the uptake of $A\beta_{1-42}$ by macrophages. A β was added to PMA-differentiated macrophage-like U937 cells incubated with either Alzheimer's or normal CSF (50%) and after 24 h the supernatant was collected and analysed by immunoblotting. In all cases, the supernatant from cells incubated with Alzheimer's CSF contained more $A\beta_{1-42}$ than supernatant from cells incubated with normal CSF (Fig. 4). In two of three cases, there was no remaining A β detected in culture supernatants of cells incubated with normal CSF samples (Fig. 4). A large fraction of the $A\beta_{1-42}$ present in supernatants from cultures incubated with Alzheimer's CSF was present as SDS-resistant oligomers. The oligomers ranged from around 10 kDa to just under 25 kDa, corresponding to two to five A β monomers (one monomer ~4.5 kDa) (Fig. 4). When compared with corresponding non-extracellular chaperone-supplemented cultures, the addition of extracellular chaperones decreased the amount of $A\beta_{1-42}$ remaining in the supernatant (Fig. 4). Furthermore, for the Alzheimer's CSF supplemented cultures, the addition of extracellular chaper-

Fig. 4 AD CSF inhibits $A\beta_{1-42}$ uptake by macrophage-like cells. Western blot image of $A\beta_{1-42}$ remaining in the CSF containing supernatant after incubation with PMA-differentiated macrophage-like U937 cells. Exogenous $A\beta_{1-42}$ was added to CSF to give a concentration of 1 μM . The CSF was diluted 1:1 in AIM V and added to cells and incubated for 24 h. At the conclusion of the incubation, samples were centrifuged for 10 min at $3,000\times g$. The supernatant was removed and analysed by 15% SDS PAGE and

subsequent immunoblotting using WO2 (anti-A β monoclonal antibody). Bound specific antibodies were detected with HRP-conjugated sheep anti-mouse Ig antibody followed by enhanced chemiluminescence (ECL) with Supersignal West Pico Chemiluminescent Substrate. Individual CSF samples are labelled 1–3 (see first paragraph in Results section). Results shown are representative of at least two independent experiments

ones reduced the fraction of supernatant A β found in SDS-resistant oligomers (Fig. 4).

Discussion

It is thought that an increase in A β production and/or a reduced rate of clearance of A β from extracellular central nervous system fluids may be contributing factors in the development of Alzheimer's pathology (Iwata et al. 2005). One or more types of soluble A β species are likely to be responsible for the neurotoxicity associated with Alzheimer's disease, as the pathology is correlated with elevated levels of A β in the brain (Naslund et al. 2000) but does not correlate well with the location of insoluble A β plaques (Terry et al. 1991). In the current study, we have shown that Alzheimer's CSF is toxic when incubated with neuroblastoma cells of human origin (SH-SY5Y) (Fig. 1). The toxicity can, at least in part, be suppressed by the addition of extracellular chaperones at concentrations equivalent to those found in normal human CSF. The extracellular chaperones clusterin, haptoglobin and α_2 m are present in normal human CSF at 2 μ g/ml, 0.5–2 μ g/ml and 1–3.6 μ g/ml, respectively (Murphy et al. 1988; Sobek and Adam 2003; Biringer et al. 2006). The mechanism by which Alzheimer's CSF is toxic and the means by which extracellular chaperones protect cells from Alzheimer's CSF cytotoxicity is unknown. Extracellular chaperones, such as clusterin, have been shown to protect cells from a range of stresses (Wilson and Easterbrook-Smith 2000). Clusterin and α_2 m have both been shown to protect cells from A β toxicity (Boggs et al. 1996; Fabrizi et al. 2001) and TNF α -mediated cell death (Humphreys et al. 1997; Arandjelovic et al. 2007). α_2 m can also protect cells by binding to and trapping proteases (Ikari et al. 2001). It has been demonstrated that both clusterin and Hp protect cells from oxidative stress-induced toxicity (Schwochau et al. 1998; Melamed-Frank et al. 2001). It is possible that one or more of these potential mechanisms of toxicity are being blocked by the action of the extracellular chaperones. Collectively, this suggests that these extracellular chaperones may play a cytoprotective role in Alzheimer's disease.

We next examined the ability of CSF to protect neuroblastoma cells from the toxicity of added exogenous A β_{1-42} . Under the conditions tested, normal CSF protected cells from A β_{1-42} toxicity, while Alzheimer's CSF did not (Fig. 3). This is consistent with the fact that the *in vitro* formation of amyloid fibrils by A β is inhibited by normal human CSF but less so by Alzheimer's CSF (Ono et al. 2005). Taken together, these results suggest that, at least in the samples tested, there is a fundamental change in the composition of Alzheimer's CSF that compromises its ability to inhibit the aggregation and toxicity of A β . In

the current study, this may relate to the differences found in the levels of the extracellular chaperones clusterin, α_2 m and Hp. The lower total protein concentration of Alzheimer's CSF may account for the lower concentrations of extracellular chaperones in Alzheimer's CSF found in this study, suggesting that extracellular chaperones are not selectively depleted from this fluid. This is consistent with previous work showing that Alzheimer's disease patient CSF has a lower total protein concentration than normal CSF controls (Moriyama et al. 1998). In contrast, previous studies suggested that the concentration of at least one of the extracellular chaperones, clusterin, is not lower in Alzheimer's CSF (Lidstrom et al. 2001; Nilselid et al. 2006). However, while previous studies suggest that clusterin concentration does not differ in spinal fluid of Alzheimer's patients, it is possible that the differences in relative clusterin levels in spinal fluid described in the current study could be attributed to technical differences between the Western blotting technique used here and the ELISA method used previously (Lidstrom et al. 2001; Nilselid et al. 2006). It is also impossible to rule out the possibility that the differences seen in the present study could be due to the small sample size used.

In the current study using Western blotting, we show that the levels of clusterin, α_2 m and Hp are lower in samples of Alzheimer's CSF compared to normal CSF. Moreover, addition of exogenous extracellular chaperones into Alzheimer's CSF provided partial protection to neuroblastoma cells from A β toxicity. Previous studies have also shown that clusterin and α_2 M protect cells in primary rat mixed neuronal cultures from A β toxicity (Boggs et al. 1996; Du et al. 1997). Taken together, these results are consistent with the idea that, in the CSF of Alzheimer's patients, lower levels of extracellular chaperones may render the CSF less able to sequester and safely remove A β than is the case in healthy individuals. Both α_2 m and clusterin have been shown to mediate cellular A β uptake via receptor-mediated endocytosis (Hammad et al. 1997; Narita et al. 1997). This is in accordance with the theory that these extracellular chaperones play a role in sequestering and disposing of dangerously hydrophobic proteins or peptides *in vivo* (Yerbury et al. 2005a,b; Wilson et al. 2008). The current study suggests that Alzheimer's CSF is less effective at promoting the cellular uptake of exogenously added A β than control CSF and that the addition of extracellular chaperones can promote the uptake of A β_{1-42} by macrophage-like cells. This is consistent with *in vivo* studies that show that the rate of clearance of A β from the mouse brain is significantly increased by both α_2 m (Shibata et al. 2000) and clusterin (Bell et al. 2007).

In conclusion, the results presented here are consistent with a model in which extracellular chaperones bind A β and maintain its solubility, protect cells from its toxicity and

subsequently promote its removal from the brain fluid via receptor-mediated endocytosis. Results from this study suggest that Alzheimer's CSF is less able to protect cells from A β toxicity and promote removal of A β from CSF. In addition, our results suggest that increasing the concentration of extracellular chaperones in Alzheimer's brain fluid may alleviate A β toxicity and promote its removal from the brain. Thus, increasing the concentration of extracellular chaperones in the brain may represent a potential therapeutic strategy for the treatment of Alzheimer's disease.

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References

- Arandjelovic S, Dragojlovic N, Li X, Myers RR, Campana WM, Gonias SL (2007) A derivative of the plasma protease inhibitor alpha(2)-macroglobulin regulates the response to peripheral nerve injury. *J Neurochem* 103(2):694–705. doi:[10.1111/j.1471-4159.2007.04800.x](#)
- Bell RD, Sagare AP, Friedman AE, Bedi GS, Holtzman DM, Deane R, Zlokovic BV (2007) Transport pathways for clearance of human Alzheimer's amyloid beta-peptide and apolipoproteins E and J in the mouse central nervous system. *J Cereb Blood Flow Metab* 27(5):909–918
- Biringer RG, Amato H, Harrington MG, Fonteh AN, Riggins JN, Huhmer AF (2006) Enhanced sequence coverage of proteins in human cerebrospinal fluid using multiple enzymatic digestion and linear ion trap LC-MS/MS. *Brief Funct Genomic Proteomic* 5(2):144–153. doi:[10.1093/bfpg/ell026](#)
- Boggs LN, Fuson KS, Baez M, Churgay L, McClure D, Becker G, May PC (1996) Clusterin (Apo J) protects against in vitro amyloid-beta (1–40) neurotoxicity. *J Neurochem* 67(3):1324–1327
- Couratier P, Sindou P, Esclaire F, Louvel E, Hugon J (1994) Neuroprotective effects of riluzole in ALS CSF toxicity. *Neuroreport* 5(8):1012–1014. doi:[10.1097/00001756-199404000-00040](#)
- Du Y, Ni B, Glinn M, Dodel RC, Bales KR, Zhang Z, Hyslop PA, Paul SM (1997) alpha2-Macroglobulin as a beta-amyloid peptide-binding plasma protein. *J Neurochem* 69(1):299–305
- Fabrizi C, Businaro R, Lauro GM, Fumagalli L (2001) Role of alpha2-macroglobulin in regulating amyloid beta-protein neurotoxicity: protective or detrimental factor? *J Neurochem* 78(2):406–412. doi:[10.1046/j.1471-4159.2001.00419.x](#)
- French K, Yerbury JJ, Wilson MR (2008) Protease activation of α 2-macroglobulin modulates a chaperone-like action with broad specificity. *Biochemistry* 47(4):1176–1185. doi:[10.1021/bi701976f](#)
- Gong Y, Chang L, Viola KL, Lacor PN, Lambert MP, Finch CE, Krafft GA, Klein WL (2003) Alzheimer's disease-affected brain: presence of oligomeric A β ligands (ADDLs) suggests a molecular basis for reversible memory loss. *Proc Natl Acad Sci U S A* 100(18):10417–10422. doi:[10.1073/pnas.1834302100](#)
- Greenberg SM, Briggs ME, Hyman BT, Kokoris GJ, Takis C, Kanter DS, Kase CS, Pessin MS (1996) Apolipoprotein E epsilon 4 is associated with the presence and earlier onset of hemorrhage in cerebral amyloid angiopathy. *Stroke* 27(8):1333–1337
- Hammad SM, Ranganathan S, Loukinova E, Twal WO, Argraves WS (1997) Interaction of apolipoprotein J-amyloid beta-peptide complex with low density lipoprotein receptor-related protein-2 megalin—a mechanism to prevent pathological accumulation of amyloid beta-peptide. *J. Biol. Chem.* 272(30):18644–18649. doi:[10.1074/jbc.272.30.18644](#)
- Hughes SR, Khorkova O, Goyal S, Knaeblein J, Heroux J, Riedel NG, Sahasrabudhe S (1998) Alpha2-macroglobulin associates with beta-amyloid peptide and prevents fibril formation. *Proc Natl Acad Sci U S A* 95(6):3275–3280. doi:[10.1073/pnas.95.6.3275](#)
- Humphreys D, Hochgrebe TT, Easterbrook-Smith SB, Tenniswood MP, Wilson MR (1997) Effects of clusterin overexpression on TNFalpha- and TGFbeta-mediated death of L929 cells. *Biochemistry* 36(49):15233–15243. doi:[10.1021/bi9703507](#)
- Humphreys DT, Carver JA, Easterbrook-Smith SB, Wilson MR (1999) Clusterin has chaperone-like activity similar to that of small heat shock proteins. *J Biol Chem* 274(11):6875–6881. doi:[10.1074/jbc.274.11.6875](#)
- Ikari Y, Mulvihill E, Schwartz SM (2001) alpha 1-Proteinase inhibitor, alpha 1-antichymotrypsin, and alpha 2-macroglobulin are the antiapoptotic factors of vascular smooth muscle cells. *J Biol Chem* 276(15):11798–11803. doi:[10.1074/jbc.M008503200](#)
- Iwata N, Higuchi M, Saido TC (2005) Metabolism of amyloid-beta peptide and Alzheimer's disease. *Pharmacol Ther* 108(2):129–148. doi:[10.1016/j.pharmthera.2005.03.010](#)
- Kida E, Choi-Miura NH, Wisniewski KE (1995) Deposition of apolipoproteins E and J in senile plaques is topographically determined in both Alzheimer's disease and Down's syndrome brain. *Brain Res* 685(1–2):211–216. doi:[10.1016/0006-8993\(95\)00482-6](#)
- Klein WL (2002) Abeta toxicity in Alzheimer's disease: globular oligomers (ADDLs) as new vaccine and drug targets. *Neurochem Int* 41(5):345–352. doi:[10.1016/S0197-0186\(02\)00050-5](#)
- Le WD, Rowe DB, Jankovic J, Xie W, Appel SH (1999) Effects of cerebrospinal fluid from patients with Parkinson disease on dopaminergic cells. *Arch Neurol* 56(2):194–200. doi:[10.1001/archneur.56.2.194](#)
- Lichtenfels R, Biddison WE, Schulz H, Vogt AB, Martin R (1994) CARE-LASS (calcein-release-assay), an improved fluorescence-based test system to measure cytotoxic T lymphocyte activity. *J Immunol Methods* 172(2):227–239. doi:[10.1016/0022-1759\(94\)90110-4](#)
- Lidstrom AM, Hesse C, Rosengren L, Fredman P, Davidsson P, Blennow K (2001) Normal levels of clusterin in cerebrospinal fluid in Alzheimer's disease, and no change after acute ischemic stroke. *J Alzheimer's Dis* 3(5):435–442
- Mattson MP (2004) Pathways towards and away from Alzheimer's disease. *Nature* 430(7000):631–639. doi:[10.1038/nature02621](#)
- Maynard CJ, Bush AI, Masters CL, Cappai R, Li QX (2005) Metals and amyloid-beta in Alzheimer's disease. *Int J Exp Pathol* 86(3):147–159. doi:[10.1111/j.0959-9673.2005.00434.x](#)
- Melamed-Frank M, Lache O, Enav BI, Szafraneck T, Levy NS, Ricklis RM, Levy AP (2001) Structure–function analysis of the antioxidant properties of haptoglobin. *Blood* 98(13):3693–3698. doi:[10.1182/blood.V98.13.3693](#)
- Moriyama T, Kudo T, Ikura Y, Kashiwagi Y, Miyamae Y, Nakamura Y, Tanaka T, Shinozaki K, Nishikawa T, Takeda M (1998) Increased tau protein level in postmortem cerebrospinal fluid. *Psychiatry Clin Neurosci* 52(1):107–110. doi:[10.1111/j.1440-1819.1998.tb00981.x](#)
- Muchowski PJ, Wacker JL (2005) Modulation of neurodegeneration by molecular chaperones. *Nat Rev Neurosci* 6(1):11–22. doi:[10.1038/nrn1587](#)
- Murphy B, Kirszbaum L, Walker ID, d'Apice JF (1988) SP-40, 40, a newly identified normal human serum protein found in the SC5b-9 complex of complement and in the immune deposits in

- glomerulonephritis. *J Clin Invest* 81:1858–1864. doi:[10.1172/JCI113531](https://doi.org/10.1172/JCI113531)
- Narita M, Holtzman DM, Schwartz AL, Bu G (1997) Alpha2-macroglobulin complexes with and mediates the endocytosis of beta-amyloid peptide via cell surface low-density lipoprotein receptor-related protein. *J Neurochem* 69(5):1904–1911
- Naslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, Buxbaum JD (2000) Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *JAMA* 283(12):1571–1577. doi:[10.1001/jama.283.12.1571](https://doi.org/10.1001/jama.283.12.1571)
- Nilselid AM, Davidsson P, Nagga K, Andreasen N, Fredman P, Blennow K (2006) Clusterin in cerebrospinal fluid: analysis of carbohydrates and quantification of native and glycosylated forms. *Neurochem Int* 48(8):718–728. doi:[10.1016/j.neuint.2005.12.005](https://doi.org/10.1016/j.neuint.2005.12.005)
- Ono K, Noguchi M, Matsumoto Y, Yanase D, Iwasa K, Naiki H, Yamada M (2005) Cerebrospinal fluid of Alzheimer patients promotes [beta]-amyloid fibril formation in vitro. *Neurobiol Dis* 20(2):233–240. doi:[10.1016/j.nbd.2005.03.005](https://doi.org/10.1016/j.nbd.2005.03.005)
- Poon S, Rybchyn MS, Easterbrook-Smith SB, Carver JA, Wilson MR (2000) Clusterin is an ATP-independent chaperone with very broad substrate specificity that stabilizes stressed proteins in a folding-competent state. *Biochemistry* 39:15953–15960. doi:[10.1021/bi002189x](https://doi.org/10.1021/bi002189x)
- Poon S, Treweek TM, Wilson MR, Easterbrook-Smith SB, Carver JA (2002) Clusterin is an extracellular chaperone that specifically interacts with slowly aggregating proteins on their off-folding pathway. *FEBS Lett* 513(2–3):259–266. doi:[10.1016/S0014-5793\(02\)02326-8](https://doi.org/10.1016/S0014-5793(02)02326-8)
- Powers JM, Schlaepfer WW, Willingham MC, Hall BJ (1981) An immunoperoxidase study of senile cerebral amyloidosis with pathogenetic considerations. *J Neuropathol Exp Neurol* 40(6):592–612. doi:[10.1097/00005072-198111000-00002](https://doi.org/10.1097/00005072-198111000-00002)
- Schwochau GB, Nath KA, Rosenberg ME (1998) Clusterin protects against oxidative stress in vitro through aggregative and non-aggregative properties. *Kidney Int* 53(6):1647–1653. doi:[10.1046/j.1523-1755.1998.00902.x](https://doi.org/10.1046/j.1523-1755.1998.00902.x)
- Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, Holtzman DM, Miller CA, Strickland DK, Ghiso J, Zlokovic BV (2000) Clearance of Alzheimer's amyloid-ss(1–40) peptide from brain by LDL receptor-related protein-1 at the blood–brain barrier. *J Clin Invest* 106(12):1489–1499. doi:[10.1172/JCI10498](https://doi.org/10.1172/JCI10498)
- Silvestrelli G, Lanari A, Parnetti L, Tomassoni D, Amenta F (2006) Treatment of Alzheimer's disease: from pharmacology to a better understanding of disease pathophysiology. *Mech Ageing Dev* 127(2):148–157. doi:[10.1016/j.mad.2005.09.018](https://doi.org/10.1016/j.mad.2005.09.018)
- Sobek, O. and P. Adam (2003). On S. Seyfert, V. Kunzmann, N. Schwertfeger, H. C. Koch, A. Faulstich: Determinants of lumbar CSF protein concentration. *J Neurol* 250(3): 371–372. doi:[10.1007/s00415-003-0991-6](https://doi.org/10.1007/s00415-003-0991-6)
- Strauss S, Bauer J, Ganter U, Jonas U, Berger M, Volk B (1992) Detection of interleukin-6 and alpha 2-macroglobulin immunoreactivity in cortex and hippocampus of Alzheimer's disease patients. *Lab Invest* 66(2):223–230
- Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R (1991) Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol* 30(4):572–580. doi:[10.1002/ana.410300410](https://doi.org/10.1002/ana.410300410)
- Wilson MR, Easterbrook-Smith SB (2000) Clusterin is a secreted mammalian chaperone. *Trends Biochem Sci* 25(3):95–98. doi:[10.1016/S0968-0004\(99\)01534-0](https://doi.org/10.1016/S0968-0004(99)01534-0)
- Wilson MR, Yerbury JJ, Poon S (2008) Potential roles of abundant extracellular chaperones in the control of amyloid formation and toxicity. *Mol Biosyst* 4(1):42–52. doi:[10.1039/b712728f](https://doi.org/10.1039/b712728f)
- Yerbury JJ, Rybchyn MS, Easterbrook-Smith SB, Henriques C, Wilson MR (2005a) The acute phase protein haptoglobin is a mammalian extracellular chaperone with an action similar to clusterin. *Biochemistry* 44(32):10914–10925. doi:[10.1021/bi050764x](https://doi.org/10.1021/bi050764x)
- Yerbury JJ, Stewart EM, Wyatt AR, Wilson MR (2005b) Quality control of protein folding in extracellular space. *EMBO Rep* 6(12):1131–1136. doi:[10.1038/sj.embor.7400586](https://doi.org/10.1038/sj.embor.7400586)
- Yerbury JJ, Poon S, Meehan S, Thompson B, Kumita JR, Dobson CM, Wilson MR (2007) The extracellular chaperone clusterin influences amyloid formation and toxicity by interacting with prefibrillar structures. *FASEB J* 21(10):2312–2322. doi:[10.1096/fj.06-7986com](https://doi.org/10.1096/fj.06-7986com)
- Yerbury JJ, Kumita JR, Meehan S, Dobson CM, Wilson MR (2009) {alpha}2-Macroglobulin and haptoglobin suppress amyloid formation by interacting with prefibrillar protein species. *J Biol Chem* 284(7):4246–4254. doi:[10.1074/jbc.M807242200](https://doi.org/10.1074/jbc.M807242200)