Comment

Comment on: Neurotoxicity of prion peptide 106–126 not confirmed, by Beat Kunz, Erika Sandmeier, Philipp Christen

FEBS Letters 485 (1999) 65–68[1]

Gianluigi Forloni[a,*], Mario Salmona[a], Orso Bugiani[b], Fabrizio Tagliavini[b]

[a] *Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea 62, 20157 Milan, Italy*
[b] *Istituto Nazionale Neurologico 'Carlo Besta', Milan, Italy*

Received 3 December 1999

Edited by Giorgio Semenza

Our work with synthetic prion protein (PrP) peptides stemmed from previous findings that (i) the amyloid protein from patients with Gerstmann–Sträussler–Scheinker (GSS) disease (a genetic form of prion disease) is a PrP fragment whose N-terminus is located within residues 58–90 (i.e. the octapeptide repeat region) and C-terminus corresponds to residue ~150 [1,2], and (ii) the accumulation of this fragment in the brain is accompanied by neuronal degeneration and glial activation [3]. Since amyloid proteins have a high content of β-sheet secondary structure [4] – a feature which distinguishes the normal PrP (PrP^C) from disease-specific PrP isoforms (PrP^{Sc}) [5] – we synthesised a series of peptides corresponding to consecutive segments of the GSS amyloid subunit to investigate which PrP sequence is central to conformational change and amyloid formation, and whether accumulation of abnormal PrP peptides is responsible for nerve and glial cell changes found in prion diseases. These studies showed that, unlike other PrP sequences, the peptide homologous to residues 106–126 (PrP106–126) is sparingly soluble in aqueous solutions, has a high propensity to adopt β-sheet secondary structure and form amyloid fibrils, is neurotoxic, and induces hypertrophy and proliferation of astrocytes [6–9]. Drs D. Brown and H. Kretzschmar extended these studies and showed that PrP106–126 activates microglial cells which increases their oxygen radical production, becoming an important mediator of neurotoxicity [10]. It is noteworthy that these and many other effects (e.g. interaction of the peptide with cell membranes, changes of intracellular calcium levels) have been observed with the protease-resistant core of PrP^{Sc} [11–14] suggesting that the sequence comprising residues 106–126 plays a major role in the conformational conversion of PrP^C into PrP^{Sc} as well as in the pathogenesis of brain changes occurring in prion diseases.

Following our original report [6], numerous other groups have confirmed the neurotoxicity of PrP106–126 in primary cultures and cell lines (see [15] for references). PrP106–126 has also been used to evaluate the relationship between conformational state and biological activity [16] and to test neuroprotective drugs [17,18]. In these studies, a scrambled sequence of PrP106–126 as well as other PrP peptides and chemically unrelated peptides were analysed to provide adequate control conditions, as mentioned by D. Brown in the comment on Kunz's article [19]. The peptides were obtained from several different sources, both industrial (e.g. Bachem, Seven Biotech Ltd) and academic (e.g. University of Göttingen, Edinburgh, Madrid), and the possibility that a chemical contaminant with similar properties was systematically and exclusively associated with PrP106–126 seems highly improbable. If, in the unlikely case, this event could have occurred, then we believe that such a contaminant should be identified. This is an important control experiment that has been omitted by Kunz and co-workers [15] but, in our opinion, is essential to substantiate their findings.

It is not unusual to find variations in biological activity of synthetic amyloid peptides between different batches independent of their sequence. A prime example is the amyloid-β (Aβ) of Alzheimer's disease. Following the first demonstration that Aβ peptides are neurotoxic in vitro [20], contrasting results were obtained by other groups, and an entire issue of Neurobiology of Aging (Vol. 13, No. 5, 1992) was dedicated to this debate. Today, the toxicity of Aβ peptides is widely accepted, although it is common experience to find batches that do not exhibit neurotoxicity even after peptide aging. One possible explanation for this discrepancy is that different lots of the same peptide can adopt different conformations and have different aggregation properties, which may be important determinants of neurotoxicity [21]. However, the relationship between these factors is complex and only partially understood. Neurotoxicity might be due to metastable protofibrillar intermediates rather than – or in addition to – mature amyloid fibrils and an excess of fibrillogenic ability might indeed lower the neurotoxic activity of a peptide [22,23]. Consequently, the interpretation of results from cell culture studies requires extensive characterisation and knowledge of the chemicophysical properties of individual peptides. When such information is limited, it is inappropriate to derive general conclusions, particularly when a peptide was obtained from a single source. This is one of the main problems in the study by Kunz and co-workers.

A surprising fact is that Dr Kunz was unable to observe neurotoxicity using a PrP106–126 peptide obtained by Dr Kretzschmar [24] and, on the other hand, Dr Brown found some neurotoxicity using 'unfiltered' PrP106–126 provided by Kunz [19]. These results suggest differences in methodologies. However, as previously mentioned, it is important to remem-

*Corresponding author. Fax: (39)-2-39001916.
E-mail: forloni@irfmn.mnegri.it

[1] PII: S0014-5793(99)01123-0

PII: S0014-5793(99)01781-0

ber that the neurotoxicity of PrP106–126 has been successfully replicated in many laboratories throughout the world. Since Dr Kunz and co-authors are open to suggestions, we would advise them to test several commercially available PrP peptides and make the results public.

References

[1] Tagliavini, F., Prelli, F., Ghiso, J., Bugiani, O., Serban, D., Prusiner, S.B., Farlow, M.R., Ghetti, B. and Frangione, B. (1991) EMBO J. 10, 513–519.

[2] Tagliavini, F., Prelli, F., Porro, M., Rossi, G., Giaccone, G., Farlow, M.R., Dlouhy, S.R., Ghetti, B., Bugiani, O. and Frangione, B. (1994) Cell 79, 695–703.

[3] Farlow, M.R., Tagliavini, F., Bugiani, O. and Ghetti, B. (1991) in: Handbook of Clinical Neurology (De Jong, J.M.B.V., Ed.), Vol. 16, pp. 619–633, Elsevier Science, Amsterdam.

[4] Glenner, G.G. (1980) New Engl. J. Med. 302, 1333–1343.

[5] Pan, K.M., Baldwin, M., Nguyen, J., Gasset, M., Serban, A., Groth, D., Mehlhron, I., Huang, Z., Fletterick, R.J., Cohen, F.E. and Prusiner, S.B. (1993) Proc. Natl. Acad. Sci. USA 90, 10962–10966.

[6] Forloni, G., Angeretti, N., Chiesa, R., Monzani, E., Salmona, M., Bugiani, O. and Tagliavini, F. (1993) Nature 362, 543–546.

[7] Tagliavini, F., Prelli, F., Verga, L., Giaccone, G., Sarma, R., Gorevic, P., Ghetti, B., Passerini, F., Ghibaudi, E., Forloni, G., Salmona, M., Bugiani, O. and Frangione, B. (1993) Proc. Natl. Acad. Sci. USA 90, 9678–9682.

[8] Selvaggini, C., De Gioia, L., Cantu, L., Ghibaudi, E., Diomede, L., Passerini, F., Forloni, G., Bugiani, O., Tagliavini, F. and Salmona, M. (1993) Biochem. Biophys. Res. Commun. 194, 1380–1386.

[9] Forloni, G., Del Bo, R., Angeretti, N., Chiesa, R., Smiroldo, S., Doni, R., Ghibaudi, E., Salmona, M., Porro, M., Verga, L., Giaccone, G., Bugiani, O. and Tagliavini, F. (1994) Eur. J. Neurosci. 6, 1415–1422.

[10] Brown, D.R., Schmidt, B. and Kretzschmar, H.A. (1996) Nature 380, 345–347.

[11] Prusiner, S.B., McKinley, M.P., Bowman, K.A., Bolton, D.C., Bendheim, P.E., Groth, D.F. and Glenner, G.G. (1983) Cell 35, 349–358.

[12] De Armond, S.J., Gonzales, M., Mobley, W.C., Kon, A.A., Sern, H. and Prusiner, S.B. (1988) in: Alzheimer's Disease and Related Disorders. Progress in Clinical and Biological Research (Ikbal, K. et al., Eds.), pp. 601–618, Alan R. Liss, New York.

[13] Müller, W.E.G., Ushijima, H., Schröder, H.C., Forrest, J.M.S., Schatton, W.F.H., Rytik, P.G. and Heffner-Lauc, M. (1993) Eur. J. Pharmacol. 246, 261–267.

[14] Kristensson, K., Feurerstein, B., Taraboulos, A., Hyun, W.C., Prusiner, S.B. and DeArmond, S.J. (1993) Neurology 43, 2335–2341.

[15] Kunz, B., Sandmeider, E. and Christen, P. (1999) FEBS Lett. 458, 65–68.

[16] Jobling, M.F., Stewart, L.R., White, A.R., McLean, C., Friedhuber, A., Maher, F., Beyreuther, K., Masters, C.L., Barrow, C.J., Collins, S.J. and Cappai, R. (1999) J. Neurochem. 73, 1557–1565.

[17] Perovic, S., Pergande, G., Ushijima, H., Kelve, M., Forrest, J. and Muller, W.E. (1995) Neurodegeneration 4, 369–374.

[18] Perez, M., Wandosell, F., Colaco, C. and Avila, J. (1998) Biochem. J. 335, 369–374.

[19] Brown, D.R. (1999) FEBS Lett. 460, 559–560.

[20] Yankner, B.S., Duffy, L.K. and Kirshner, D. (1990) Science 250, 279–282.

[21] Soto, C., Castano, E.M., Kumar, R.A., Beavis, R.C. and Frangione, B. (1995) Neurosci. Lett. 200, 105–108.

[22] Hartley, D.M., Walsh, D.M., Ye, C.P., Diehl, T., Vasquez, S., Vassilev, P.M., Teplow, D.B. and Selkoe, D.J. (1999) J. Neurosci. 19, 8876–8884.

[23] Oda, T., Wals, P., Ostenburg, H.H., Johnson, S.A., Pasinetti, G.M., Morgan, T.E., Rozovsky, I., Stine, W.B., Snyder, S.W., Holzman, T.F. and Finch, K. (1995) Exp. Neurol. 136, 22–31.

[24] Kunz, B., Sandmeier, E. and Christen, P. (1999) FEBS Lett. 460, 561.