Comment

Comment on: Neurotoxicity of prion peptide 106–126 not confirmed

by Beat Kunz, Erika Sandmeier, Philipp Christen, *FEBS Letters* 458 (1999) 65–68

David R. Brown*

Department of Biochemistry, Cambridge University, Tennis Court Road, Cambridge CB2 1QW, UK

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The analysis of the toxicity of the abnormal form of the prion protein (PrPSc) using a cell culture model has been centred on the toxicity of a synthetic peptide based on the sequence of the human prion protein. The toxicity of this peptide was reported initially by Forloni et al. [1]. Since then a vast body of research has appeared characterising the mechanism of toxicity of this peptide, its ability to mimic the toxicity of PrPSc in culture [2] and its effect on glial cells. The recent article by Kunz et al. (1999) published in *FEBS Letters* makes the claim that the peptide has no inherent toxicity and that the only toxicity the authors found was due to HPLC chemicals not removed during purification. A detailed analysis of this paper indicates that the authors have no valid reason for their assertion.

PrP106–126 has been demonstrated to be toxic by many independent laboratories [1,3–8] including my own [9]. Unlike the work of Kunz et al. these experiments were often carried out using multiple control peptides [1] which included known toxic peptides such as those related to the β-amyloid sequence or those based on the sequence of human PrP but not toxic. In many cases the secondary structure and fibrillar nature of the peptides was studied in parallel in order to determine what physical qualities were necessary for the toxicity [10]. Such an analysis is missing from the paper of Kunz et al. Even more so, in some studies cultures of cells from PrP-deficient mice were used as a control as PrP106–126 was not toxic to these cells. If the toxicity was related to HPLC contaminants then one would expect toxicity to be the same on both cell types. The paper of Kunz et al. contains no such control experiments. It seems unlikely that HPLC contaminants could have been the cause of PrP106–126 toxicity in situations where different peptides at the same concentrations prepared with similar techniques had different toxicities [1].

The authors claim that the toxicity they report in Table 1 of their article (Authentic HPLC) is due to HPLC contamination. However, the authors do not identify what this contaminant is. This should have been a relatively simple matter given that the authors had access to mass spectroscopy techniques and know the composition of their HPLC buffer. The finding that HPLC buffer is toxic has no relevance to the work of others. Most biochemists involved with peptide synthesis are aware of the need to remove HPLC contaminants and the peptides that I have used have been analysed by synthesis and mass spectroscopy similar to those described by Kunz et al. The lack of apparent toxicity of their peptide (apparently from only two preparations) cannot be explained by the addition of toxic chemicals to their preparations.

Despite the large number of papers quoted by the authors on the toxicity of PrP106–126 the authors seem to have paid little attention to their content. The methodology used in preparing their peptides has not been used in most of the papers describing this peptide. Inducing fibril formation and spinning out the fibrils using ultracentrifugation is not necessary for the induction of a toxic nature for this peptide. Indeed, although the authors claim to induce fibrils there is no evidence that there were any fibrils in their preparations (e.g. no EM micrographs). This preparation technique seems a rather odd thing to do given that the authors then routinely filtered the fibrils from their stock solutions as described in Section 3.3 of their article. It has also been published that the peptide must consist of a mixture of aggregated and non-aggregated material in order to elicit a maximal toxic response [10]. This is something the authors appear not to take into account when carrying out their experiments. Also, although the authors confirm the purity of their peptide initially they do not, after their long and complicated treatment process, confirm how much peptide actually reaches their cultures. If not all the peptide forms fibrils before ultracentrifugation then some percentage would be lost. Also if most of the peptide does form fibrils then it is unlikely that any of their peptide would pass through their 0.2 μm filter. Therefore as this method was used for almost all the experiments in their paper then it is likely that the authors added no peptide to their cultures.

Additionally, it has been published that microglia are necessary for the toxicity of PrP106–126 [3]. However, the authors do not add microglia to their cultures to see if this enhances the toxicity of their peptide, or that of their HPLC buffer.

The cortical cell cultures used for the authors' Fig. 1 appear to have been very low density. Additionally their cultures appear to be full of debris typical of poor and dying cultures. The authors' claim to have used methods used by other laboratories. However, despite quoting my own work the method they describe is not the same as the one that I used or have continued to use either in the laboratory of Kretzschmar [3] or in my own [9]. Indeed, a major problem with the paper is the total lack of analysis of the cultures used. It is possible that the cultures prepared by Kunz et al. contained no or very few neurones. Also, as microglia are necessary for the toxicity of PrP106–126 the authors need to show that their cultures contain microglia before the toxicity of PrP106–126 can be brought into doubt. A further point, given the low density of the cultures, is that the majority of the experiments the authors describe were carried out in 96 well plates. I doubt

*Fax: (44) (1223) 333345.
E-mail: drb33@cam.ac.uk

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Table 1
Peptide toxicity to cerebellar cell cultures determined by MTT assay

	% Untreated control value
No peptide	100 ± 2
PrP106–126	53 ± 4
Control PrP106–126 (scrambled)	98 ± 2
Kunz et al. PrP106–126	78 ± 3
Kunz et al. PrP106–126 (filtered)	98 ± 3

Peptides were applied for 5 days at 80 μM. The methods used were as previously described [3]. Mean and S.E.M. for three experiments with all peptides in each experiment (three determinations each). The experiments were done in 24 well plates.

that the authors could produce statistically meaningful results with such small measurements. As regards the measurements themselves, there is no indication in the paper as to how long exposure to the peptide was for (i.e. how many days). Many experiments are reported as (Data not shown) or have low, statistically minuscule, *n* values (i.e. 2 or 1).

The final problem with this work is that the authors do not present all the data they have. I was contacted by the first author during the time these experiments were being carried out. I agreed to test their peptide for toxicity. I received their peptide and was able to carry out a small number of experiments on cerebellar cells in parallel with peptide of my own. I used filtered and unfiltered samples of their peptide and found that, like Kunz et al., the filtered peptide was not toxic. However, the non-filtered peptide was toxic (see Table 1). The toxicity was much less than that of my own peptide. Above all else this result suggests that there was a problem in the methodology employed by Kunz et al. in assessing the toxicity of PrP106–126. Clearly, the ability to filter out the toxicity of a peptide solution with a 0.2 μm filter indicates that the toxicity of their peptide was not due to an HPLC contaminant.

In summary, Kunz et al. have not demonstrated that PrP106–126 is not toxic. Their paper lacks the kinds of controls and assays necessary to reach such a conclusion. There are many possible explanations for their results, none of which have been explored by the authors, the simplest of which could be that insufficient peptide, in the right conformation, reaches their cells for a toxic effect.

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