IMPLICATION OF UNDIGESTED GLUTEN PEPTIDES ON NEUROLOGICAL DISORDERS: STUDY OF MOLECULAR MECHANISMS IN EXPERIMENTAL MODELS OF EPILEPSY

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Background: Some nutrients are able to influence neuronal functions and synaptic plasticity by acting on molecular systems that are vital for maintaining cognitive function and essential for brain development. Diet is linked to physiological but also pathological conditions and what we consume seems to have significant implications for the brain. For example, gluten related disorders (GRD) are frequently associated with neurological and psychiatric manifestations (Julian et al., 2018). In particular, people with epilepsy diagnosed with celiac disease (CD) seems to be characterized by intractable seizure. In these patients, gluten restriction diet has ensued in a reduction of both seizure frequency and antiepileptic medications (Bashiri, H., et al., 2016). However, the molecular mechanisms that associates GRD and epileptogenesis are yet unknown. The gliadin peptides 31-43(p31-43) (involved in innate immunity) and 57-68(p57-68) (the immunodominant peptide that induce the adaptive immunoresponse in CD), are two of the main gliadin peptides that remain undigested by the intestine (Shan et al., 2002). In particular, p31-43has been shown to induce toxicity both in in vitro and *in vivo* tissues obtained from patients with CD (Maiuri, L. et al., 1996, Ciclitira, P. J. & Ellis, H. J., 1998). The aim of our study was to examine the neurotoxic effects of the gliadin peptides p31-43and p57-68in *in vivo* and in vitro models of kainate-induced-epilepsy, in order to characterize the molecular mechanisms that correlates GRD and epileptogenesis.

Methods: For *in vivo* experiments, we used C57/B mice intraperitoneally injected either with kainate or with p31-43or their combination and then observed for 90 min in order to assess latency, type and duration of epileptic seizures. For in vitro experiments, we used organotypic hippocampal slices exposed to kainate (5μM) for 24h, a classical model of temporal epilepsy (Morin-Brureau et al., 2013), alone or in combination with p31-43or p57-68. Neuronal cell death was evaluated with propidium iodide fluorescence. We then analyzed the electrophysiological responses of p31-43alone on spontaneous excitatory synaptic currents and the role of transglutaminases.

Results: The administration of p31-43exacerbates the number and the duration of seizures induced by kainate in *in vivo* experiments and worsens the CA3injury induced by kainate in in vitro experiments, showing a correlation between p31-43and epilepsy. Surprisingly, 1h preincubation of p31-43exacerbates the kainate-induced toxicity not only in CA3but also in CA1region of rat organotypic hippocampal slices. Moreover, the electrophysiological responses of p31-43on CA3pyramidal neurons showed an increase of spontaneous excitatory synaptic currents and in the total number of evoked APs in neurons, indicating an enhanced neuronal excitability. Furthermore, p31-43significantly increased the expression of TG2and TG6, indicating that its neurotoxic effect involve the transglutaminase family.

Conclusions: Our study associates the toxic effects of gluten to epilepsy and evidence mechanisms of induced toxicity by the gliadin peptide p31-43that could consider gluten free diet as a possible therapeutic strategy for intractable seizure in patients affected by GRD.