β-Methylamino-L-alanine (L-BMAA) induces neuronal injury via kinase-dependent mechanisms in rat primary neuronal cultures

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Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that leads to the death of patients within 3-5 years after diagnosis. The only drug (riluzole) that exists prolongs the lifespan of patients by a few months. Although the pathophysiology of **ALS** is not completely understood, it shows close similarities with other neurodegenerative disease like **ALS-PDC** (**Amyotrophic lateral sclerosis/parkinsonism-dementia complex**) characterized by clusters of dysfunctional proteins within neurons. Indeed, several studies have pointed to the potential role of dietary exposure to the cyanobacteria-derived non-amino acid, **β-Methylamino-L-alanine** (**L-BMAA**) as a possible risk factor for ALS or ALS/PDC. Furthermore, post-mortem analyses indicate the presence of L-BMAA in the brains of patients that suffered from non-genetic progressive neurodegenerative disease, including ALS.

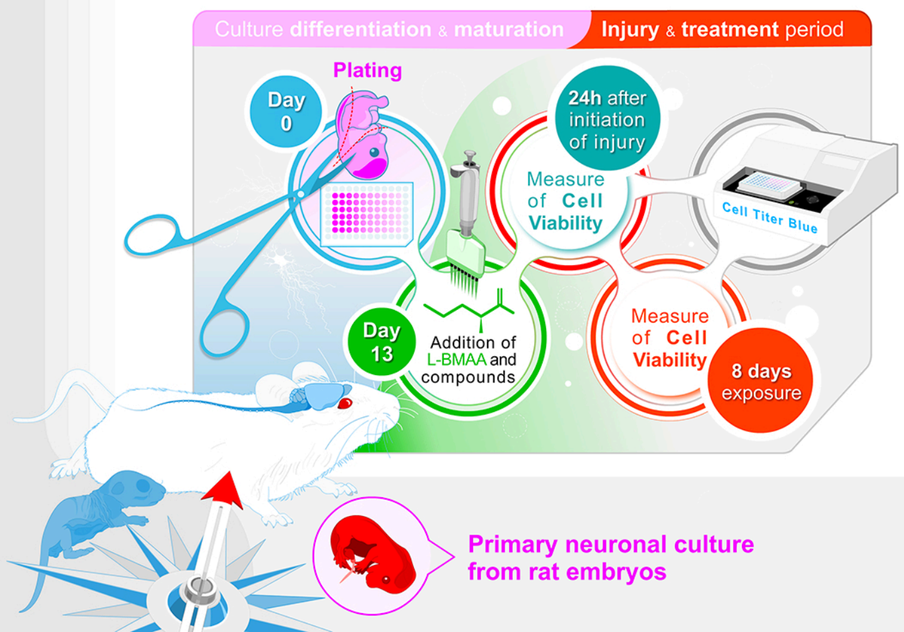
Objective

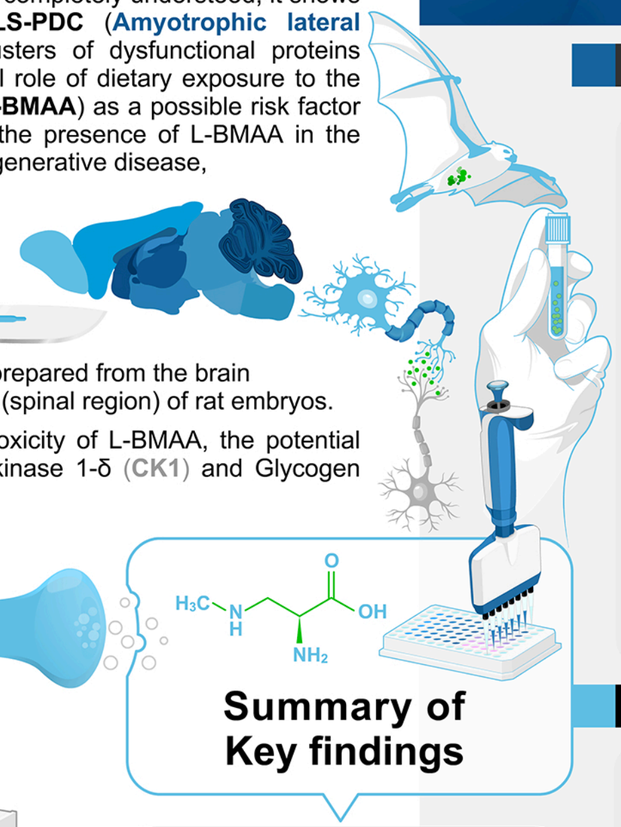
The present study has two objectives:

1. evaluate the neurotoxicity of L-BMAA in primary neuronal cultures prepared from the brain (cortical, mesencephalic and hippocampal regions) and spinal cord (spinal region) of rat embryos.
2. assess the putative role of protein phosphorylation in the neurotoxicity of L-BMAA, the potential neuroprotective effect of two candidate protein kinases (casein kinase 1-δ (CK1) and Glycogen synthase kinase-3 (GSK3) via the use of their respective inhibitors.

Material and Methods

Experimental design: cellular model of brain damage





Summary of Key findings

L-BMAA shows an **enhanced neurotoxicity** towards spinal motor neurons compared to other neuronal populations

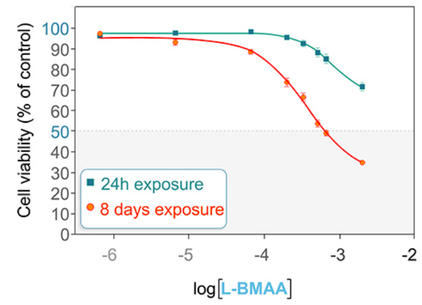
The extent of **L-BMAA-induced neuronal death** is dependent on the exposure duration

L-BMAA neurotoxicity is mediated by **protein phosphorylation mechanisms** involving protein kinases such as casein **kinase 1-δ** or **Glycogen synthase kinase-3**

Results

Figure 1:

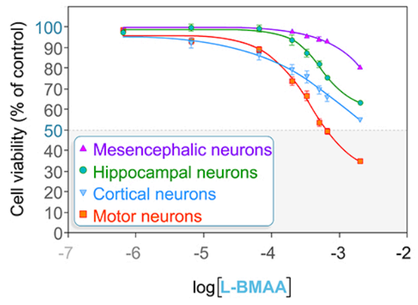
Effect of exposure duration on the neurotoxicity of L-BMAA to spinal motor neurons in culture



24h and **8 days** exposure with **L-BMAA** produced a dose-dependent reduction of the cell viability in cultures of spinal motor neurons. **Neuronal injury was markedly enhanced in 8 days as compared to 24h exposure.**

Figure 2:

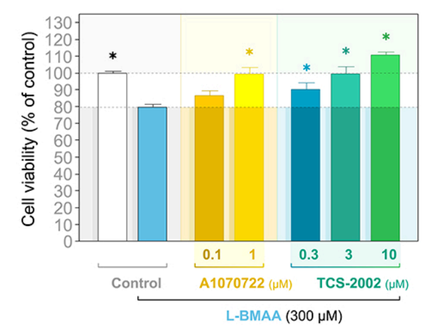
Comparison of L-BMAA-induced cell death in different types of neurons in culture



8 days exposure with **L-BMAA** produced a dose-dependent neurotoxicity in different population types of neurons.
The degree of susceptibility towards L-BMAA of the different types of neurons was in the following order:
Spinal motor neurons > **Cortical neurons** > **Hippocampal neurons** > **Mesencephalic neurons**.

Figure 3:

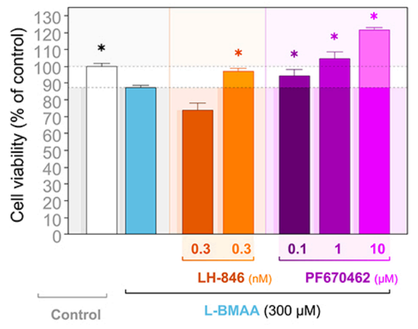
Involvement of GSK3 pathway in the neurotoxicity of L-BMAA



GSK3 inhibitors (**A1070722** and **TCS-2002**) interfered in a dose-dependent manner with the neurotoxicity of **L-BMAA** after 8 days exposure. **Full inhibition was observed at the highest tested doses.**

Figure 4:

Involvement of CK1 pathway in the neurotoxicity of L-BMAA



CK1 inhibitors (**LH-846** and **PF670462**) interfered in a dose-dependent manner with the neurotoxicity of **L-BMAA** after **8 days exposure**. **Full inhibition was observed at the highest tested doses.**