Anti-inflammatory activity of polyarginine nanocapsules loaded with astaxanthin

Jimena Cordero-Machuca[1, 3], Rodrigo Naves[2], Felipe Oyarzún Ampuero[1,3]

[1]Department of Pharmaceutical Science and Technology, University of Chile, Dr. Carlos Lorca 964, Independencia, Santiago, Chile
[2] Immunology Program, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile, Av. Independencia 1027, Santiago, Chile
[3]Advanced Center of Chronic Diseases (ACCDiS), Sergio Livingstone 1007, Independencia, Santiago, Chile

jimena.cordero@ug.uchile.cl

Astaxanthin (AST) is a carotenoid obtained from natural sources that has been reported to have an extremely strong antioxidant, anti-inflammatory, and neuroprotective activity [1]. Therefore, AST might have protective effects in neuroinflammatory diseases such as multiple sclerosis, an inflammatory immune-mediated disease of the CNS characterized by demyelination and neurodegeneration. However, the therapeutic application of AST is hindered by its low aqueous solubility and poor stability [2]. In order to improve AST stability and bioavailability as well as its potential application in neuroinflammation, we have developed polyarginine (PARG) nanocapsules (NCs) loaded with AST. PARG is a cationic polymer that has been reported to enhance interaction and transport through cell membranes [3]. The NCs were synthesized using a modified solvent displacement method described previously by Lollo (2017) [4]. PARG was tested at different concentrations (0.06 - 0.25 mg/mL). This strategy allowed us to obtain stable PARG-NCs in the range of 157 - 204 nm, with PDI values (0.1 - 0.2), and positive zeta potential between 33.8 and 57.5 mV, confirming the adherence of polyarginine on the oily droplet´s surface. The positive Zeta potential is relevant since the NCs are designed to be mucoadhesive and to promote absorption of AST through cell membranes. To determine cytotoxicity of PARG-NCs, rat astrocytes were incubated with NCs synthetized with different concentration of PARG (0.06, 0.125, and 0.25 mg/ml) and at volumes of 5-25% of cell culture medium volume. After 48 hours, cell viability was assessed by flow cytometry. The results showed that cell viability was higher than 90% in the presence of PARG-NCs at concentrations of 0,06 mg/mL and 0,125 mg/mL PARG and at volume lower than 15% (Figure 1). To evaluate the *in vitro* anti-inflammatory activity of AST-PARG-NCs, interferon (IFN)-gamma activated rat astrocytes were treated with PARG-NCs loaded with 20 µM AST and the expression of the glial fibrillary acidic protein (GFAP), a commonly used marker of astroglial activation, was determined by flow cytometry. The preliminary results suggest that activated astrocytes treated with PARG-NCs containing 20 µM AST exhibited lower expression of GFAP than control cells (Figure 2). Taking together, these results indicate that PARG-NCs are safe and that they might be useful as nanosystem for AST delivery with potential anti-inflammatory application in neuroinflammatory diseases. To the best of our knowledge, this is the first time that anti-inflammatory activity of PARG-NCs loaded with AST is reported using astrocytes.

References

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Figures

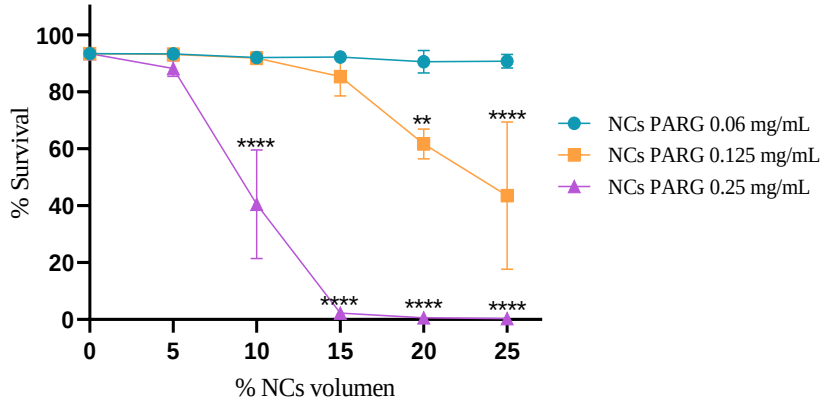

Figure 1. Survival percentage of astrocytes incubated for 48 h with NCs PARG (0.06, 0.125, 0.25 mg/mL of PARG) at volumes of 5 to 25% in relation to cell culture medium volume. **** $p < 0.0001$ vs NCs with 0.06 mg/mL PARG. The results are shown as mean of n = 3

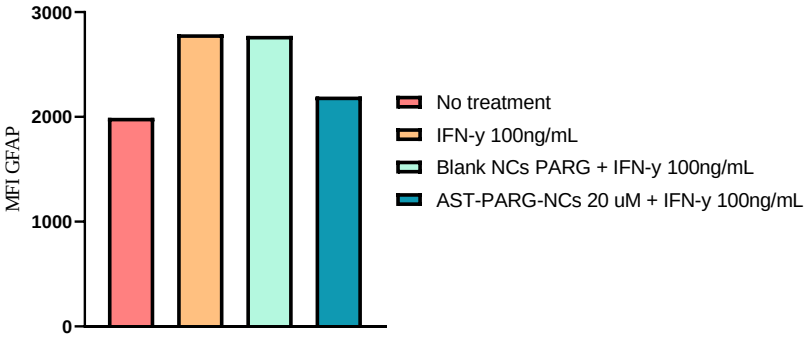

Figure 2. GFAP expression in rat astrocytes after inflammatory activation with 100 ng/mL IFN-gamma and treated with Blank NCs PARG (vehicle) or AST-PARG-NCs 20 µM.