SIMULTANEOUS ACTIVATION OF MU AND DELTA OPIOID RECEPTORS REDUCES ALLODYNIA AND ASTROCYTIC CONNEXIN 43IN AN ANIMAL MODEL OF NEUROPATHIC PAIN

Nunzio Vicario[1], Lorella Pasquinucci[2], Federica Maria Spitale[1], Santina Chiechio[3], Rita Turnaturi[2], Filippo Caraci[4], Daniele Tibullo[5], Roberto Avola[6], Rosario Gulino[7], Rosalba Parenti[7], Carmela Parenti[8]

[1]1Dept of Biomedical and Biotechnological Sciences, Section of Physiology, University of Catania, Catania - Italy, [2]Dept of Drug Sciences, Section of Medicinal Chemistry, University of Catania, Catania - Italy, [3]Dept of Drug Sciences, Section of Pharmacology and Toxicology, University of Catania, Oasi Research Institute - IRCCS, Troina, Catania - Italy, [4]Dept of Drug Sciences, Section of Pharmacology and Toxicology, University of Catania, Oasi Research Institute - IRCCS, Troina, Catania - Italy, [5]Dept of Biomedical and Biotechnological Sciences, Section of Biochemistry, University of Catania, Catania - Italy, [6]Dept of Biomedical and Biotechnological Sciences, Section of Biochemistry, University of Catania, Catania - Italy, [7]Dept of Biomedical and Biotechnological Sciences, Section of Physiology, University of Catania, Catania - Italy, [8]Dept of Drug Sciences, Section of Pharmacology and Toxicology, University of Catania, Oasi Research Institute - IRCCS, Troina, Catania - Italy

Neuropathic pain is a chronic condition triggered by lesions to the somatosensory nervous system in which pain stimuli occur spontaneously or as pathologically amplified responses. In this scenario, the exchanges of signaling molecules throughout cell-to-cell and cell-to-extracellular environment communications play a key role in the transition from acute to chronic pain. As such, connexin 43(Cx43), the core glial gap junctions and hemichannels forming proteins, is considered a triggering factor for disease chronicization in the central nervous system (CNS). Drugs targeting μ opioid receptors (MOR) are currently used for moderate to severe pain conditions, but their use in chronic pain is limited by the tolerability profile. δ opioid receptors (DOR) have become attractive targets for the treatment of persistent pain and have been associated with the inhibition of pain sustaining factors. Moreover, it has been shown that simultaneous targeting of MOR and DOR led to an improved pharmacological fingerprint. The multitarget antinociceptive agent LP2, a benzomorphan-based compound with a (R/S)-2-methoxy-2-phenylethyl group as N-substituent emerged for its high MOR (K_i= 1.08nM) and DOR (K_i= 6.6nM) affinity. In vitro and *in vivo* evidences showed that LP2is a MOR/DOR agonist (IC_{50}^{MOR}= 21.5nM and IC_{50}^{DOR}= 4.4nM; ED_{50}:0.9mg/kg i.p.) showing the pharmacodynamic profile of a potent multitarget MOR/DOR antinociceptive ligand.

Herein, we aimed to investigate the potential of simultaneous MOR/DOR targeting in a model of unilateral sciatic nerve chronic constriction injury (CCI). Body weight and behavioural assessment of mechanical allodynia were examined at 0 (before surgery), 3, 5, 11, 16and 21days post ligatures (dpl). Conventional neuropathological analysis was performed on transverse spinal cord sections analyzing neuronal and astroglial phenotype in CCI-vehicle and CCI-LP2treated rats. Moreover, a reductionistic in vitro model of excitotoxic pulse was analyzed using DRG-derived neurons and spinal cord derived astrocytes.

Our data showed that LP2treatment significantly ameliorated mechanical allodynia since the early phase of the disease up to 21dpl. We additionally showed that LP2prevented CCI-induced reactive astroglial expression of Cx43(1.2±0.4CCI-LP2vs. 5.2±0.8CCI-vehicle, fold change over sham-vehicle) and the proportion of pro-apoptotic cells (4.0±1.1% CCI-LP2vs. 8.6±0.9% CCI-vehicle) in the spinal dorsal horn at 21dpl. These evidences were confirmed in vitro, founding that conditioned media of picrotoxin-stimulated neurons increased reactive astrogliosis and that LP2treatment, beyond exerting a sustained neuroprotective effect, reduced astroglial Cx43-based coupling (1.9±0.3picrotoxin+LP2vs. 4.6±0.9picrotoxin+vehicle, fold change over control cultures).

Our findings relate to the reduction of neurological deficits upon peripheral injury and involve neuro-glial axis; concurrently, reduced Cx43levels at a central level are crucial to ameliorate the behavioral impairment from the early stages to the chronic phases of the disease. These findings are paving the way for the development of new analgesic compounds with MOR/DOR profile as a new pharmacological strategy for the treatment of neuropathies and to prevent central sensitization.