PAM-2 decreases neuropathic pain in mice and modulates chemokine/cytokine production in human microglial cells

Hugo R. Arias[a,b], Randall L. Davis[b], Daniel J. Buck[b], Carla Ghelardini[c] , Elena Lucarini[c], Dina Manetti[d], Maria Novella Romanelli[d], and Lorenzo Di Cesare Mannelli[c]

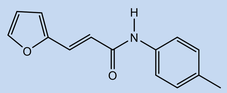
[a,b] Department of Pharmacology and Physiology, Oklahoma State University Center for Health Sciences, Tahlequah[a] and Tulsa[b], OK, USA.

[c,d] Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA), Section of Pharmacology and Toxicology[c] and Section of Pharmaceutical and Nutraceutical Sciences[d], University of Florence, Florence, Italy

INTRODUCTION

In light of the present opioid crisis, there is an urgent need to identify novel, non-opioid drugs to treat chronic pain. Drugs targeting α7-nicotinic acetylcholine receptors (α7 nAChRs) are of particular interest. α7 nAChRs are expressed in different cells within both the peripheral and central nervous system, and in glial cells, including astrocytes and microglia. Agonists with high selectivity for α7 nAChRs have demonstrated beneficial effects on neuropsychiatric and neurological diseases by decreasing neuroinflammation. However, little is known about the beneficial effect of α7-selective positive allosteric modulators (α7-PAMs) on these diseases, including chronic pain. The overall objective of this work is to evaluate 1) the anti-neuropathic pain activities of PAM-2, a highly selective α7-PAM (Arias et al., 2016) in a mouse model of drug-induced neuropathic pain; and 2) the potentiating activity of PAM-2 on (-)-nicotine-induced inhibition of chemokine/cytokine release from human microglial cells.

PAM-2



METHODS

The anti-neuropathic pain activity of PAM-2 was tested by using the oxaliplatin-induced neuropathic pain animal model (Cavaletti et al., 2001). Mice (n = 10/condition) were intermittently administered (i.p.) with 2.4 mg/kg oxaliplatin to develop neuropathic pain. Mice were then administrated (p.o.) with vehicle, 1 or 3 mg/kg PAM-2, in an acute or sub-chronic (7 and 14 days) manner. The pain threshold was subsequently determined by the cold plate test at 0, 15, 30, 45, and 60 min after treatment. Methyllycaconitine (MLA; 6 mg/kg), an α7-selective antagonist, was used to determine receptor selectivity. Data were analyzed by ANOVA, followed by Bonferroni's pairwise comparisons, and presented as mean ± SEM.

Cytokine/chemokine expression was induced in human C20 microglial cells by exposing to interleukin-1β (IL-1β; 20 ng/ml) for 18 h. Cells were stimulated with IL-1β alone or in combination with PAM-2 (1-100 μM) and/or (-)-nicotine (3 μM). The levels of pro-inflammatory chemokine/cytokines in the culture media were measured by ELISA. These *in vitro* experiments are preliminary, therefore, statistical analyses have yet to be performed.

RESULTS

PAM-2 inhibited neuropathic pain in mice

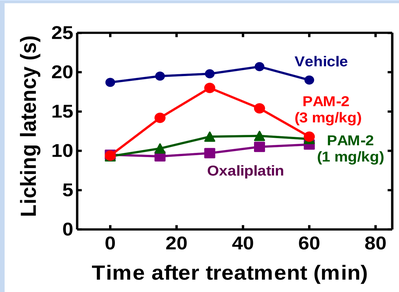


Fig. 1. Neuropathic pain was first induced with oxaliplatin. PAM-2 was then administrated (p.o.), and the response to a thermal stimulus was subsequently evaluated by the cold plate test. The licking latency was recorded at 0, 15, 30, 45, and 60 min after treatment. Bonferroni's tests indicated that PAM-2 at 3.0 mg/kg, but not 1.0 mg/kg, decreased oxaliplatin-induced neuropathic pain (vs vehicle) during the 15-45 min period ($P < 0.01$).

The anti-neuropathic activity elicited by PAM-2 is mediated by α7 nAChRs

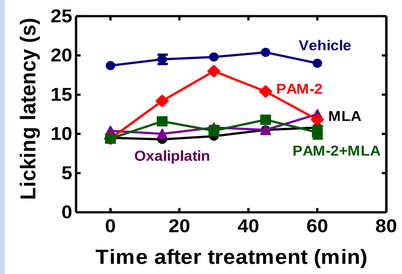


Fig. 2. Neuropathic pain was first induced by oxaliplatin. MLA (6.0 mg/kg) was administered (i.p.) 15 min before 3.0 mg/kg PAM-2 (p.o.), and the response to a thermal stimulus subsequently evaluated by the cold plate test. The licking latency was recorded at 0, 15, 30, 45, and 60 min after treatment. Bonferroni's tests indicated that MLA inhibited the anti-neuropathic pain effect of PAM-2 during the 15-45 min period ($P < 0.01$), compared to PAM-2-treated animals, whereas MLA did not change oxaliplatin-induced neuropathic pain.

The anti-neuropathic activity elicited by PAM-2 is increased after sub-chronic treatment

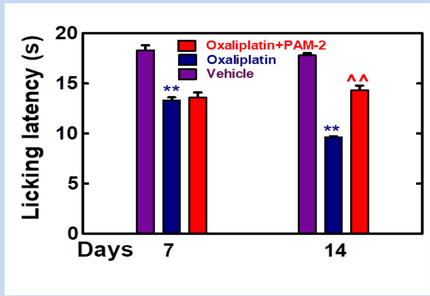


Fig. 3. Mice were co-treated with 1.0 mg/kg PAM-2 (i.e., ineffective dose) and oxaliplatin for 7 and 14 days, and cold plate tests were performed 24 h after the last administration. Bonferroni's test indicated that PAM-2 decreased oxaliplatin-induced neuropathic pain after 14 days, but not after 7 days. **$P < 0.01$ vs vehicle; ^^$P < 0.01$ vs oxaliplatin).

PAM-2 differentially modulated chemokine/cytokine production in human microglia

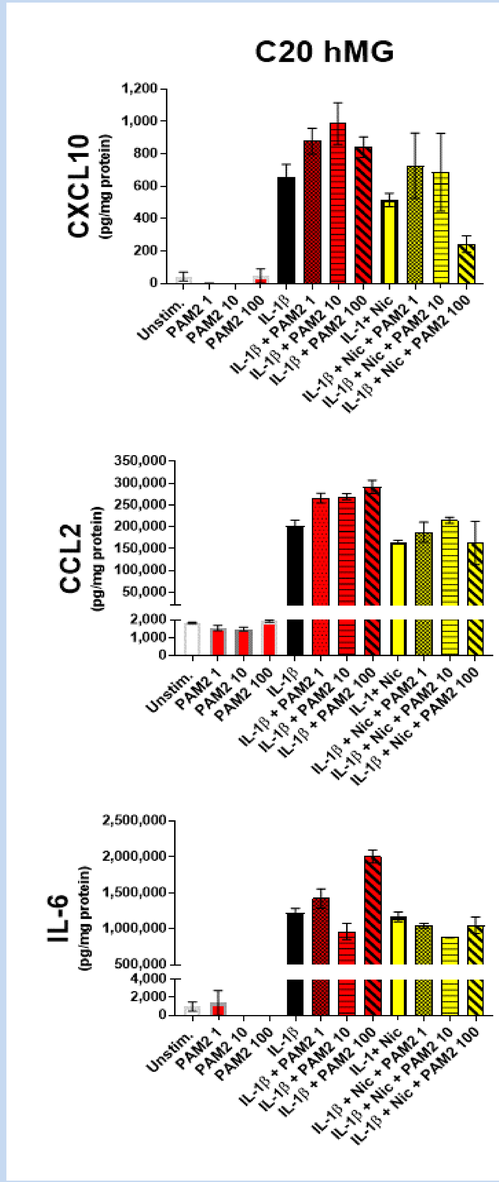


Fig. 4. Human C20 microglial cells were cultured (in 24-well plates) in the presence of vehicle, PAM-2 (1-100 μM), IL-1β (20 ng/ml), IL-1β + PAM-2, IL-1β + nicotine (3 μM), or IL-1β + nicotine + PAM-2, for 18 h. Chemokine/cytokine levels in the culture medium were measured by ELISA.

CONCLUSIONS

1. PAM-2 inhibits neuropathic pain in mice
2. PAM-2 seems to potentiate IL-1β-induced chemokine/cytokine production in human microglial cells
3. PAM-2 seems to potentiate the *inhibitory* effect of (-)-*nicotine* on IL-1β-induced CXCL10 production
4. PAM-2, and α7-PAMs in general, should be pursued as potential therapeutic agents to treat neuropathic pain

FUTURE DIRECTIONS

1. Identify the molecular and cellular targets involved in the anti-neuropathic pain actions of PAM-2, and related compounds.
2. Further investigate the modulatory effects of PAM-2 on inflammatory signaling in astrocytes and microglia.

REFERENCES

1. Arias, H.R., et al., (2016) Positive allosteric modulators of α7 nicotinic acetylcholine receptors affect neither the function of other ligand- and voltage-gated ion channels and acetylcholinesterase, nor β-amyloid content. *Int. J. Biochem. Cell Biol.* **76**, 19–30.
2. Cavaletti, G., et al., (2001) Effects of different schedules of oxaliplatin on the peripheral nervous system of the rat. *Eur. J. Cancer* **37**, 2457-2463.

ACKNOWLEDGEMENTS

Funded in part by OSU-CHS (H.R.A.)