SOCIETÀ ITALIANA DI FARMACOLOGIA

# MODELLO PER INVIO RELAZIONE DI FINE PERIODO

**NOME E COGNOME**: _____GIADA_____AMODEO _____

**UNIVERSITÀ**: ____UNIVERSITA' DEGLI STUDI DI MILANO_____

**DIPARTIMENTO (in caso di borsa per soggiorno all'estero specificare l'ente presso cui si è svolta la ricerca)**: _____DIP. DI SCIENZE FARMACOLOGICHE E BIOMOLECOLARI_____

**TUTOR (in caso di borsa per soggiorno all'estero specificare il tutor dell'ente presso cui si è svolta la ricerca): _____PROF.SSA PAOLA SACERDOTE_____**

**TIPOLOGIA DI BORSA RICEVUTA**: _____BORSA SIF-MSD 2017_____

**TIPOLOGIA DI RELAZIONE (es.: metà periodo o finale):** _____FINE PERIODO____

**TITOLO DELLA RELAZIONE:** _____ROLE OF A NEW FAMILY OF CHEMOKINES, THE PROKINETICINS, IN A MOUSE MODEL OF CHEMOTHERAPY-INDUCE PERIPHERAL NEUROPATHY_____

**RELAZIONE:**

Chemotherapy Induced Peripheral Neuropathy (CIPN) is one of the most frequent and disabling forms of neuropathic pain and it represents a dose-limiting side effect of several antineoplastic agents. Up to now there are no valid treatments to cure it and the main consequence is dose reduction or cessation, thus leading to cancer-related mortality increase.

Even if mechanisms at the basis of CIPN have not been fully understood yet, improvements have been made by using animal models. Studies have suggested that CIPN is a multifactorial pathology in which several factors are known to play. These include: mithocondrial toxicity and oxidative stress, ion channels alterations and neuroinflammatory processes (Ferrier et al., 2013). Pathological interactions between neurons, immune inflammatory cells, glial and microglia cells are the basis of its development, and in this network cytokines and chemokines have a pivotal role in the initiation and persistence of pain (Sacerdote et al., 2013; Sommer & Kress 2004).

Among chemokines, Prokineticins have an important role in immunomodulation, inflammation and in the development of different experimental neuropathic conditions such as chronic constriction injury (CCI-model) (Lattanzi et al., 2015) and diabetic neuropathy (STZ-model) (Castelli et al., 2016). In humans and rodents Prokineticin-1 (PK1) and Prokineticin-2 (PK2) (Negri et al., 2007) are ligands of two metabotropic receptors known as PKR1 and PKR2 (Masuda et al., 2002; Soga et al., 2002). PK1, PK2 and their receptors are expressed by the immune cells (i.e. granulocytes, macrophages, lymphocytes) and in the main stations of pain transmission (i.e. brain, spinal cord, dorsal root ganglia, nerves).

It has been demonstrated that PK2 acts on macrophages and induces a pro-inflammatory profile by stimulating IL-1 and IL-12 production and by inhibiting the IL-10 release (Franchi et al., 2008), which can lead to reduced nociceptive thresholds to thermal and mechanical stimuli (Negri et al., 2006; Giannini et al., 2009). Furthermore, in some nervous regions associated with pain, there is a colocalization of PKRs and TRPV receptors, and a lot of neurons that respond to PK2 express and release CGRP and substance P

(Vellani et al., 2006; De Felice et al., 2012). Moreover, our previous data demonstrated a key role of the PK system in development of painful symptoms in several mouse models of neuropathic pain (CCI/STZ-model). Given these premises, it can be hypothesized that this system may be an important player also in CIPN. For this reason the present study has the purpose to investigate the involvement of Prokineticin system in the development and maintenance of CIPN.
CIPN was induced in mice by the administration of two different chemotherapeutic agents commonly used in oncology: Bortezomib (BTZ), the first of a new class of agents that inhibit the 26s complex of proteasome favouring apoptosis of cancer cells , and Vincristine (VCR), the most neurotoxic drug among Vinka Alkaloids. Although the drugs have different mechanisms of action, they are both related to CIPN development.
Male mice 9 weeks old of the C57BL/6J strain were used in the experiments. Based on the most common protocols used in literature, CIPN was induced in mice through the administration of Bortezomib, intraperitoneally injected (i.p.) 0.4mg/kg, 3 times a week for 4 consecutive weeks (cumulative dose, c.d., 4,8 mg/kg), or Vincristine 0,1mg/kg, i.p.injected daily for 14 consecutive days (c.d. 1,4 mg/kg) (Boehmerle et al., 2014; Kiguchi et al., 2008). Control mice were injected with the vehicle.
Allodynia, a painful response to innocuous stimuli, is one of the most frequent symptom of CIPN both in humans and rodent models and for this reason mechanical and thermal allodynia were monitored respectively by using Dynamic Plantar Aesthensiometer and Acetone Drop Test. Since in patients sensory alterations associated to CIPN are multiple, we also monitored in the animals the development of thermal hyperalgesia, an increased pain perception of painful stimuli, by using Plantar test. All behavioural tests were performed before and after neuropathic induction by the two chemotherapeutic agents.
Both BTZ and VCR were able to induce rapid and dose-related painful response to mechanical and thermal stimuli. Hypersensitivity was maintained for the whole duration of chemotherapeutic treatments. In order to unveil the involvement of the prokineticin system in CIPN we applied a pharmacological approach, using PC1, a non peptidic antagonist of PK receptors. In presence of a well established hypersensitivity, we started a therapeutic treatment with PC1: 14 or 7 days after the 1st BTZ or VCR administration, respectively. The antagonist was subcutaneously injected at the dose of 150µg/Kg, twice daily until the end of the chemotherapy schedule.

PC1 was able to rapidly contrast the neuropathic condition. In fact, chronic treatment with PC1 was able to significantly ameliorate all tested painful symptoms in mice treated either with BTZ or VCR . At the end of BTZ/PC1 and VCR/PC1 treatments, 28 and 14 days after the 1$^{st}$ BTZ/VCR administration, mice were sacrificed and tissues collected for the biochemical analysis.

PK system expression levels were analysed in the main stations involved in nociceptive transmission: sciatic nerve, Dorsal Roots Ganglia (DRG) and spinal cord. At the end of chemotherapy schedule, in these tissues, f both in BTZ and VCR-mice it was present a generally upregulation of the PK system (PK2 and its receptors).
In BTZ-mice, 28 days after the first BTZ administration, we observed a significantly increase of PK2 in all nervous tissues while the PKR1 and PKR2 levels were up-regulated only in DRG and spinal cord.
PC1-treatment was able to normalize PK2 levels in all tissues analyzed and, to significantly decrease levels of PKR1 in both DRG and spinal cord, while PKR2 levels were normalized by PC1 treatment only in spinal cord. In VCR-mice, 14 days after the first VCR administration, all PK system appears altered both in DRG and spinal cord, and PC1- treatment was able to significantly decrease PK2, PKR1 and PKR2 levels.

Since Prokineticins are considered as a bridge between neuronal components and immune cells, and it was demonstrated that cytokine modulation is necessary for experimental neuropathic pain treatment (Sacerdote et al., 2013), we analyzed, in the same nervous tissues, expression levels (RT-PCR) of different cytokines which are known to be involved in pathological pain processes. Peripheral nervous system (DRG and sciatic nerve) of BTZ-mice showed a generally pro-inflammatory profile, characterized by high levels of TNF-α, IL-6 and IL-1β. In central nervous system (spinal cord), we measured high IL-1β levels and low IL-10 levels while we did not observe alterations in TNF-α nor IL-6. PC1 administration was able to normalize altered cytokine levels in all analyzed tissues. Also VCR-mice showed a pro-inflammatory profile both in the peripheral and central nervous system, with higher levels of IL-1β, IL-6 and TNF-α in DRG and spinal cord and lower levels of IL-10 only in DRG. PC1 treatment was able to restore a correct cytokine balance, decreasing pro-inflammatory cytokines levels and increasing IL-10 levels.

Since it is known that the recruitment and infiltration of macrophages in DRG plays a key role in the development of CIPN and that PK2 is able to induce macrophage to migrate and to acquire a pro-inflammatory phenotype, and given the presence of high levels of cytokines that are commonly produced by these cells, we decided to evaluate expression levels of CD68 (Cluster of Differentiation 68) which is routinely used as a marker of inflammation with the involvement of macrophages (Chistiakov et al., 2017) and of TLR4 (Toll Like Receptor 4) that is frequently increased in inflammatory conditions. Bortezomib induced an upregulation of CD68 expression levels in all nervous tissue and also TLR4 expression in BTZ-mice significantly differed from CTR. The antagonist treatment decreased CD68 and TLR4 expression levels in sciatic nerve, DRG and spinal cord. Also VCR treatment was able to significantly increase TLR4 and CD68 levels in DRG and spinal cord; PC1 treatment decreased TLR4 levels in both tissues and CD68 only in DRG.

To assess the presence of a possible tissue damage in the CINP models, we tested mRNA levels of Activating Transcription Factor (ATF3), an adaptive response gene whose activity is usually regulated by stressful stimuli. DRG, sciatic nerve and spinal cord of CIPN-mice were characterized by an increase in ATF3 expression, indicating the presence of tissue injury and/or cell stress since it is known that ATF3 expression levels are physiologically very low in nervous tissues (Hunt et al., 2012). PC1 treatment was able to reduce altered levels of ATF3 both in sciatic nerve and spinal cord.

Furthermore, in the perspective of translating our results to the clinic, we measured the Prokineticin 2 protein levels in serum samples of CIPN-mice, because it can give us an idea of the general inflammation present in mice using blood, an accessible tissue also in human. In fact, up to now there are no clinical markers used for the diagnosis of CIPN, which is assessed mainly by self-reported pain scale and questionnaires, that are highly personal. A biochemical marker could be useful in order to have an objective parameter, which could be associated to pain scale. Our results show how, at the end of chemotherapy schedule (28 and 14 days after 1st BTZ or VCR treatment, respectively), PK2 protein levels increase in serum samples of CIPN-mice.

These results indicate that Prokineticin system is largely involved in the development and maintenance of CIPN induced by both BTZ and VCR, and that its antagonism, through the administration of PC1, is effective in contrasting the painful condition associated with chemotherapeutic treatments as well as promoting protection from neuroinflammation.

**References:**


- Boehmerle W, Huehnchen P, Peruzzaro S, Balkaya M, Endres M. (2014); “Electrophysiological, behavioral and histological characterization of paclitaxel, cisplatin, vincristine and bortezomib-induced neuropathy in C57Bl/6 mice.” Sci Rep. 18;4:6370
- Castelli M., Amodeo G., Negri L., Lattanzi R., Maftei D., Gotti C., Pistillo F., Onnis V., Congu C., Panerai A.E., Sacerdote P., Franchi S.; (2016) *Antagonism of the Prokineticin System Prevents and Reverses Allodynia and Inflammation in a Mouse Model of Diabetes.* PLoS One
- Chistiakov D.A., Killingsworth M.C., Myasoedova V.A., Orekhov A.N., Bobryshev Y. (2017); “CD68/macrosialin: not just a histochemical marker”. Laboratory Investigation 97, 4–13.
- De Felice M, Melchiorri P, Ossipov MH, Vanderah TW, Porreca F, Negri L, (2012); *"Mechanisms of Bv8-induced biphasic hyperalgesia: increased excitatory transmitter release and expression"*. NeurosciLett. 521:40-5.
- Ferrier J, Pereira V, Busserolles J, Authier N, Balayssac D, (2013); *"Emerging trends in understanding chemotherapy-induced peripheral neuropathy".* Curr Pain Headache Rep 17: 364.
- Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL; (2009) *Sex, gender, and pain: a review of recent clinical and experimental findings.* Pain**.** 10(5):447-85.
- Franchi S, Giannini E, Lattuada D, Lattanzi R, Tian H, Melchiorri P, Negri L, Panerai AE, Sacerdote P, (2008); *"The prokineticin receptor agonist Bv8 decreases IL-10 and IL-4 production in mice splenocytes by activating prokineticin receptor-1"*. Bmc Immunology 9:60.
- Giannini E, Lattanzi R, Nicotra A, Campese AF, Grazioli P, Screpanti I, Balboni G, Salvadori S, Sacerdote P, Negri L, (2009); *"The chemokine bv8/prokineticin 2 is up-regulated in inflammatorygranulocytes and modulatesinflammatorypain".* ProcNatlAcadSci USA 106:14646-51

- Hunt D, Raivich G, Anderson PN. (2012); “Activating transcription factor 3 and the nervous system”. Front Mol Neurosci 14;5:7
- Ingim
- Kiguchi N., Maeda T., Kobayashi Y., Kondo T., Ozaki M., Kishioka S.(2008); “The critical role of invading peripheral macrophage-derived interleukin-6 in vincristine-induced mechanical allodynia in mice”. Eur J Pharmacol. 592(1-3):87-92.
- Lattanzi R, Maftei D, Marconi V, Florenzano F, Franchi S, Borsani E, Rodella LF, Balboni G, Salvadori S, Sacerdote P, Negri L. (2015) Prokineticin 2 upregulation in the peripheral nervous system has a major role in triggering and maintaining neuropathic pain in the chronic constriction injury model. Biomed Res Int. 2015;2015:301292
- Masuda Y, Takatsu Y, Terao Y, Kumano S, Ishibashi Y, Suenaga M, Abe M, Fukusumi S, Watanabe T, Shintani Y, Yamada T, Hinuma S, Inatomi N, Ohtaki T, Onda H, Fujino M, (2002); *"Isolation and identification of eg-vegf/prokineticins as cognate ligands for two orphan g-protein-coupled receptors".* BiochemBiophys Res Commun 293: 396-402.
- Negri L, Lattanzi R, Giannini E, Melchiorri P, (2006); "*Modulators of pain: Bv8 and prokineticins".* CurrNeuropharmacol. 4:207-15.
- Negri L, Lattanzi R, Giannini E, Melchiorri P, (2007); "Bv/8 prokineticinproteins and their receptors". Life Sci. 81, 1103-1116.
- Sacerdote P, Franchi S, Moretti S, Castelli M, Procacci P, Magnaghi V, Panerai AE, (2013); *"Cytokine Modulation is Necessary for Efficacious Treatment of Experimental Neuropathic Pain"*. J NeuroimmunePharmacol. 8: 202-11.
- Soga T, Matsumoto S, Oda T, Saito T, Hiyama H, Takasaki J, Kamohara M, Ohishi T, Matsushime H and Furuichi K, (2002); *"Molecular cloning and characterization of prokineticin receptors"*. Biochimica et BiophisycaActa 1579, p. 173–179
- Sommer C. and Kress M.; (2004) *Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia.* Neurosci Lett., 361:184–187.
- Vellani V, Colucci M, Lattanzi R, Giannini E, Negri L, Melchiorri P, McNaughton PA, (2006); *"Sensitization of transient receptor potential vanilloid-1 by the prokineticin receptor agonist Bv8".* J Neurosci 26:5109-5116.