Fractalkine: multiple strategies to counteract glutamate receptors activation leading to neuroprotection

CX3CL1 protects neurons from Glu excitotoxicity both in vitro and in vivo models with mechanisms fully dependent on AR (Lauro et al., 2008, 2010; Cipriani et al., 2011) and partially dependent on AR (Rosito et al., 2014). Indeed CX3CL1, acting on microglia, is able to increase extracellular adenosine derived from released adenosine triphosphate (ATP), since this effect is abolished in the presence of specific inhibitor of ectonucleotidases (AOPCP) but not by the inhibitor of equilibrative transport (NBPT1) (Lauro et al., 2008, 2010). Upon Glu toxic challenge, we have shown that CX3CL1 is able to increase Glu removal from synaptic cleft by enhancing excitatory amino acid transporter GLT-1 expression and function, specifically on astrocytes, with a mechanism that depends on AR activation (Catalano et al., 2013). Moreover, CX3CL1 can modulate AMPA receptor activity by reducing AMPA current, via AR action (Lauro et al., 2008): this might lead to a reduction of Ca²⁺ flow through AMPA receptor and AMPA-mediated NMDA receptor activation.

CX3CL1 effect against Glu excitotoxicity requires also the activity of AR, whose genetic or pharmacological inactivation is able to reduce CX3CL1 neuroprotection. This mechanism is independent from GluRs activity, but represents another aspect of the neuroprotective mechanism driven by CX3CL1 that involves microglia-astrocytes crosstalk and that leads to the release of neuroprotective molecules such as CXCL16 and CCL2 (Rosito et al., 2012, 2014).

Experimental evidence suggests that NMDARs, due to their high Ca²⁺ permeability and conductance properties, are mainly responsible for uncontrolled increase of neuronal intracellular Ca²⁺ and consequent cell death. However, depending on subcellular distribution and receptor subunits composition, NMDARs stimulation may exert opposite effects on neurons: the activity of synaptic NR1/NR2A/NR2B-containing NMDARs provides neuroprotection while the activation of extrasynaptic NR1/NR2B-containing NMDARs is responsible for neurotoxicity. In particular, the activation of synaptic NR2A containing NMDAR induces cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) activity with a concomitant increased expression of neuroprotective brain-derived neurotrophic factor (BDNF); in contrast, the overactivation of extrasynaptic NR2B containing NMDAR stimulates a dominant CREB shut-off pathway, which inhibits BDNF expression and leads to excitotoxicity (Hardingham and Bading, 2010).

Interestingly, our recent work shows that CX3CL1 modulates NMDA-mediated synaptic transmission in the hippocampal CA1 region through the activation of AR and the consequent release from glial cells of D-serine, the co-agonist of NR2A/NMDAR (Scianni et al., 2013). Thus, we decided to investigate the action of CX3CL1 on NMDA-mediated neurotoxicity and, differently from what reported for Glu excitotoxicity, we found that CX3CL1 is able to contrast specific NMDA induced excitotoxicity with a mechanism that depends on AR activity and extracellular D-serine. In line with these results, D-serine is able to counteract NMDA, but not Glu excitotoxicity. Moreover, both CX3CL1 and D-serine are able to phosphorylate cyclic-AMP response element-binding protein (CREB) with a mechanism involving...
the expression of A2Ar (Lauro et al., 2015).

This is the first demonstration of a functional interaction between NMDAR and A2Ar with the purpose of modulating protective action. CX3CL1 modulates NMDAR effects in an A2Ar-dependent way, by potentiating neurotransmission and by counterbalancing the neuronal death induced by NMDAR activation through the selective potentiation of synaptic D-serine sensitive NRI/NMDARs, thus contributing to the enhancement of “CREB on” activation pathway. Nevertheless, in hippocampal cultures obtained from mice that do not express A2Ar, D-serine alone is not sufficient to induce neuroprotection or CREB phosphorylation, suggesting that in CX3CL1 neuroprotection A2Ar also acts by regulating the activity of NMDAR independently of D-serine. It should be considered that different populations of A2Ar have different localizations and functions: one possibility is that A2Ars expressed by glial cells are mostly responsible for the release of D-serine and those expressed on post-synaptic neurons modulate NMDARs activity. Moreover, when D-serine is degraded by the enzyme D-amino acid oxidase (DAAO), there is only a partial block of CREB phosphorylation, suggesting that other pathways triggered by CX3CL1 act synergistically with A2Ar to counteract NMDA-induced excitotoxicity. One of these pathways might be the BDNF/TrkB signaling: we showed that CX3CL1 increases BDNF expression and TrkB phosphorylation in hippocampal cultures, both events possibly linked to CREB phosphorylation and neuroprotection (Lauro et al., 2015).

All together, these data corroborate the idea that CX3CL1 engages different AR subtypes on neighboring cells to counteract excitotoxicity, depending on the GluRs activation; nevertheless, it has to be considered that both in acute and chronic neurodegenerative disorders, high level of Glu in the brain leads to the activation not only of NMDA receptors but also of other GluRs such as AMPA and mGlu receptors. In this contest, it might be interesting to analyze if CX3CL1 is able to activate different pathways in case of overactivation of specific AMPA or mGlu receptors to better clarify its role as a neuroprotective and neuromodulator molecule. What is clear is that CXCCL1, after the direct action on CX3CR1 expressed on microglia, counteracts GluRs activation by promoting an intense dialogue among neurons, microglia and astrocytes that cooperate to promote neuroprotective mechanisms. I conjectured that, upon excitotoxic insults, damaged neurons release CX3CL1 which acts locally, by inducing the release of different soluble factors, including adenosine, that in turn act on cells in close proximity in order to orchestrate a broad spectrum of protective activities. In this view, I want to highlight the role played by CX3CL1 as as a modulator of the GluRs, that adopting alternative strategies, drives neuroprotective action and represents an interesting endogenous self-protective mechanism initiated from neurons to counteract brain damage.

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Accepted: 2015-05-15
doi: 10.4103/1673-5374.162697 http://www.nrronline.org/
Lauro C (2015) Fractalkine: multiple strategies to counteract glutamate receptors activation leading to neuroprotection. Neural Regen Res 10(8):1214-1215.

References
Cardona AE, Piro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, Huang D, Kidd G, Dombrowski S, Dutta R, Lee JC, Cook DN, Jung S, Lira SA, Littman DR, Ransohoff RM (2006) Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci 9:917-924.
Catalano M, Lauro C, Cipriani R, Chece G, Ponzetta A, DiAngelantonio S, Ragozzino D, Limatola C (2013) CX3CL1 protects neurons against excitotoxicity enhancing GELT-activity on astrocytes. J Neuroimmunol 263:75-82.
Chapman GA, Moores K, Harrison D, Campbell CA, Stewart BR, Stribos PJ (2000) Fractalkine cleavage from neuronal membranes represents an acute event in the inflammatory response to excitotoxic brain damage. J Neurosci 20:RC87.
Cipriani R, Villa F, Chece G, Lauro C, Paladini A, Micotti E, Perego C, De Simoni MG, Fredholm BB, Eusebi F, Limatola C (2011) CX3CL1 is neuroprotective in permanent focal cerebral ischemia in rodents. J Neurosci 31:16327-16335.
Hardingham GE, Bading H (2010). Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. Nat Rev Neurosci 11:682-696.
Harrison JK, Jiang Y, Chen S, Xia Y, Maciejewski D, McNamara RK, Streit WJ, Salafranca MN, Adhkari S, Thompson DA, Botti P, Bacon KB, Feng L (1998) Role for neurally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. Proc Natl Acad Sci U S A 95:10896-10901.
Lauro C, Catalano M, Di Paolo E, Chece G, de Costanzo I, Trettel F, Limatola C (2015) Fractalkine/CX3CL1 engages different neuroprotective responses upon selective glutamate receptor overactivation. Front Cell Neurosci 8:472.
Lauro C, Cipriani R, Catalano M, Trettel F, Chece G, Brusadini V, Antonilli L, van Rosoinen J, Eusebi F, Fredholm BB, Limatola C (2010) Adenosine A1 receptors and microglial cells mediate CX3CL1-induced protection of hippocampal neurons against Glu-induced death. Neurropsychopharmacology 35:1550-1559.
Lauro C, Di Angelantonio S, Cipriani R, Sobrero F, Antonilli L, Brusadini V, Ragozzino D, Limatola C (2008) Activity of adenosine receptors type 1 is required for CX3CL1-mediated neuroprotection and neuro modulation in hippocampal neurons. J Immunol 180:7590-7596.
Rosito M, Deforio C, Limatola G, Trettel F (2012) CXCL16 orchestrates adenosine A3 receptor and MCP-1/CCL2 activity to protect neurons from excitotoxic cell death in the CNS. J Neurosci 32:3154-3163.
Rosito M, Lauro C, Chece G, Porzia A, Monaco L, Mainiero F (2014) Transmembrane chemokines CX3CL1 and CXCL16 drive interplay between neurons, microglia and astrocytes to counteract pMCAO and excitotoxic neuronal death. Front Cell Neurosci 8:193.
Scianni M, Antonilli L, Chece G, Cristalli G, Di Castro MA, Limatola C, Maggi L (2013) Fractalkine (CX3CL1) enhances hippocampal N-methyl-D-aspartate receptor (NMDAR) function via D-serine and adenosine receptor type A2 (A2AR) activity. J Neuroinflammation 10:108.