Neuroprotection by Bexarotene in Mice Subjected to Focal Cerebral Ischemia Involves Modulation of the Immune System

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Stroke is a devastating disease associated with high morbidity and mortality worldwide (Mozaffarian et al., 2015). Nevertheless, all the neuroprotective approaches tested to date have failed to translate in the clinical setting and the only pharmacological treatment currently available is thrombolysis, though its use is limited given the strict eligibility criteria and the risk of adverse effects such as haemorrhagic transformation.
Recent evidence has highlighted the neuroprotective potential of immunomodulatory strategies, based on polarization of myeloid cells towards non-inflammatory, beneficial phenotypes (Cuartero et al., 2013; Amantea et al., 2015). Given the role of retinoid X receptors (RXR) in myeloid cells differentiation and polarization, here we have explored the neuroprotective potential of the RXR agonist bexarotene in mice subjected to focal cerebral ischemia.

A single dose of bexarotene (5 or 25 mg/kg, i.p., upon reperfusion) significantly reduced cerebral infarct volume, oedema and neurological impairments produced by transient (30 min) middle cerebral artery occlusion (MCAo) in mice. The most effective dose of the rexinoid (25 mg/kg, i.p.) induced a 75% reduction of the infarct volume evaluated by cresyl violet staining of coronal brain slices 48h after reperfusion. This effect was associated with a significant reduction of the blood brain barrier rupture assessed 2 hours after reperfusion by Evans blue leakage. Bexarotene exerted neuroprotection with a wide time-window, being effective when administered up to 4.5 hours after the insult.
Immunofluorescence analysis revealed that bexarotene increases brain recruitment of macrophages and neutrophils, with a peak of infiltration 48 hours after the insult. Interestingly, the percentage of polarization of these cells toward the M2-like (CD11b+/Ym1+) and N2 (LY-6G+/Ym1+) phenotype is significantly elevated by the drug as compared to vehicle-injected animals. This was coincident with a higher density of Ly-6G/Ym1-immunopositive N2 neutrophils in the spleen of ischemic mice treated with the rexinoid. The effect of bexarotene on systemic inflammation is confirmed by its ability to inhibit spleen atrophy induced by the ischemic insult.
The improvement of histological outcomes, as well as the ability of bexarotene to revert MCAo-induced spleen atrophy, was antagonised by BR1211, a pan-RXR antagonist, or by the selective PPARγ antagonist BADGE, highlighting the involvement of the RXR/PPARγ heterodimer in the beneficial effects exerted by the drug.

In conclusion, our findings demonstrate that due to its peripheral immunomodulatory effects, consisting in the polarization of myeloid cells toward non-inflammatory (M2 and N2) phenotypes, the RXR agonist bexarotene may be effectively repurposed for the acute therapy of ischemic stroke.

Amantea et al. (2015) *Front Neurosci.* 9:147.
Cuartero et al. (2013) *Stroke.* 44, 3498-508.
Mozaffarian et al. (2015) *Circulation.* 131, 434-41.