GPER pharmacotherapy in ischemic stroke

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Experimental studies indicate that estrogen typically, but not universally, has a neuroprotective effect in experimental stroke. While clinical stroke incidence is lower and acute brain injury tends to be milder, females appear to experience worse medium- to long-term outcomes after stroke. Estrogen has been assumed to provide post-stroke neuroprotection entirely *via* classical estrogen receptors although an alternative target is the recently decribed G Protein-coupled Estrogen Receptor (GPER).

Unlike classical estrogen receptors, the GPER appears to be expressed throughout the brain, blood vessels and leukocytes to a similar extent in males and females, and so it could potentially play a role in estrogen-mediated neuroprotective effects in diseases such as stroke. Important questions therefore include whether the neuroprotection provided by endogenous estrogen occurs *via* GPER signalling, and/or if GPER modulation could represent a novel direction for stroke therapy. In addition, dampening of the immune system after stroke leaves the patient more susceptible to infections (a major cause of post-stroke mortality beyond the acute phase), but whether sex and/or GPER acivity might influence this phenomenon is still unclear.

Following cerebral ischemia-reperfusion resulting from monofilament-induced occlusion of the middle cerebral artery in intact female mice, GPER antagonist treatment (G-15) augments infarct volume to a level equivalent to that seen in ovariectomised (OVX) females and in males. In addition, treatment with the GPER agonist (G-1) reduces infarct volume and neurological deficit after ischemia-reperfusion in OVX females (an effect which can be blocked with coadministration of G-15), but G-1 has no effect in intact females. Tamoxifen (a GPER agonist) treatment also provides neuroprotection in OVX mice in a G-15-sensitive manner, in association with reduced infiltration of T lymphocytes and neutrophils into the ischemic hemisphere. Thus, greater post-ischemic neuroprotection appears to occur in intact females due to the full activation of GPER by endogenous estrogen, an effect that cannot be enhanced with the addition of a GPER agonist.

By contrast, following permanent cerebral ischemia there is no effect of sex, OVX or G-15 treatment on infarct volume, emphasising the importance of the reperfusion phase in attaining estrogen/GPER-mediated neuroprotection in females. GPER expressed on circulating immune cells may be an important target of GPER therapy.

Surprisingly, GPER agonist treatment worsens infarct volume and functional outcomes in male mice post-stroke, in association with an accelerated expression of the key apoptotic protein, cleaved caspase-3. Conversely, the GPER antagonist reduces infarct volume and improves functional outcomes in males, whether given before or after stroke.

When between-sex differences in infarct size are eliminated to facilitate testing for differences in some systemic consequences of stroke (by utilising permanent cerebral ischemia), the severity of lung bacterial infection is bimodal in females but overall tends to be less than in males, in association with a decreased spleen weight and higher mortality at 24 h. GPER inhibition significantly increases the severity of bacterial infection in females.

Our key data currently indicate that GPER activation contributes to estrogen-mediated neuroprotection (and possibly lung infections) following cerebral ischemia-reperfusion, and that GPER agonists, tamoxifen and G-1, can improve stroke outcome following surgical menopause. By contrast in males, G-1 worsens post-stroke brain injury whereas the GPER antagonist, G-15, has a neuroprotective effect – potentially by interfering with aldosterone-mediated GPER activation. Future therapies for acute stroke could therefore exploit the modulation of GPER activity in a sex-specific manner. More study is particularly needed to elucidate whether GPER modulation is effective following stroke in older females.