# TRANSFORMING GROWTH FACTOR-β1 PROTECTS RAT CORTICAL NEURONS AGAINST EARLY AND LATE β-AMYLOID TOXICITY

Filippo Caraci[1], Agata Copani[1], Giuseppe Battaglia[2], Ferdinando Nicoletti[2,3], Maria Angela Sortino[4]

[1]Dept. of Pharmaceutical Sciences, University of Catania ; [2]I.N.M. Neuromed, Pozzilli, Italy, [3]Dept. of Human Physiology and Pharmacology, University of Rome ''La Sapienza''; [4]Dept. of Experimental and Clinical Pharmacology, University of Catania

Transforming growth factor β1 (TGF-β1) is a pleiotropic cytokine, which is known to exert a neuroprotective role in Alzheimer's disease. Estrogens enhance TGF-β1 secretion in rat cortical astrocytes and the medium collected from estrogen-treated astrocytes is protective when transferred to cultured neurons challenged with β-amyloid (Aβ) (1). Several mechanisms have been proposed to explain the neuroprotective action of TGF-β1 against Aβ-induced neurotoxicity, such as cell cycle inhibition or the increased expression of anti-apoptotic proteins. Nevertheless the specific molecular pathways involved in these phenomena are presently unknown.

We examined the neuroprotective properties of TGF-β1 in pure cultures of rat cortical neurons challenged with the toxic Aβ fragment (25–35). When applied to mature pure rat cortical neurons, 25 μM Aß (25-35) (in the presence of 10 μM MK-801 + 30 μM DNQX) induced the apoptosis of about 40-50% of the total neuronal population at 24 hours. TGF-β1 (10 ng/ml), similarly to the cell-cycle inhibitor flavopiridol (0.3 μM), was able to rescue about 85-90% of Aβ-treated neurons when added 1 hour before the peptide. Interestingly, a similar extent of protection was observed when TGF-β1 was added 6 hours after Aβ, an experimental condition in which flavopiridol was inactive. These data suggest a mechanism other than cell cycle inhibition in the neuroprotective effect of TGF-β1 during the late phase of Aβ toxicity. A down-regulation of the Wnt pathway, with ensuing GSK3β activation, is known to occur late in Aβ-induced neuronal apoptosis (2). Western blot analysis showed that TGF-β1 prevented the activation of GSK3β, the increase in tau phosphorylation and the down-regulation of ß-catenin levels induced by Aß at 24 hours. TGF-β1 per se promoted the inactivation of GSK3β via the PI3k/Akt pathway. The selective inhibitor of the PI3k/Akt pathway, LY294002 (10 μM), significantly reduced the neuroprotective effects of TGF-β1 by preventing the inhibition of the cell cycle and the inactivation of GSK3β induced by TGF-β1 in Aβ-treated neurons.

We conclude that TGF-β1 both inhibits the cell cycle and rescues the Wnt signaling in Aβ-treated neurons via the PI3k/Akt pathway, thus affecting both the early and late phase of Aβ toxicity.

REFERENCES

1) Sortino M.A., Chisari M., Merlo S., Vancheri C., Caruso M., Nicoletti F., Canonico P.L.,Copani A. (2004) Endocrinology 145: 5080– 5086.

2) Caricasole A, Copani A, Caraci F, Aronica E, Rozemuller A.J., Caruso A, Storto M, Gaviraghi G, Terstappen GC, Nicoletti F. (2004) Journal of Neuroscience, 24: 6021-7.