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**Ischemia-induced apoptosis in the mammalian brain crucially correlates with long-lasting phosphorylation of c-Jun, suppression of ATF-2 and expression of the death-inducing ligands CD95-Ligand and TRAIL.**

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Neuronal apoptosis plays an important role in the pathogenesis of cerebral ischemia, but the underlying mechanisms remain to be defined. Neuronal programmed cell death was studied in an established rat model of transient focal ischemia that resulted in a defined pattern of ischemic brain damage. The expression of the death-inducing ligands (DILs) CD95-Ligand, TRAIL and TNF- $\alpha$ , and of the transcription factor c-Jun in its active, phosphorylated form, was examined in the apoptotic compartments of the postischemic brain. Between 3 h and 5 days following ischemia, CD95-Ligand, TRAIL and TNF- $\alpha$  were upregulated and neuronal c-Jun was phosphorylated at residue serine-73. Treatment of human neuron-derived neuroblastoma cells with recombinant TRAIL, TNF- $\alpha$  or an agonistic CD95 antibody caused death of the majority of the neurons, with TRAIL being the most potent cytotoxic factor. This suggests that the neuronal expression of TRAIL, CD95-L and TNF- $\alpha$  mediates ischemic cell death in the mammalian brain.

JNK/SAPK control the activity of c-Jun and ATF-2 and the transcription of c-Jun. Their activation is a downstream event of the DIL-induced cascades. Recent data indicate that the promoter of the CD95-L gene is activated in response to stimulation of AP-1 activity by the JNK/SAPK pathway. I found that JNK/SAPK activity increased significantly in the postischemic brain. Also, the constitutively expressed ATF-2 protein disappeared in the apoptotic compartments. Moreover, ATF-2 suppression paralleled the long-lasting phosphorylation of c-Jun and the expression of CD95-L. Thus, the results of the present study indicate the existence of a positive autoregulatory loop between DILs and the transcription factor c-Jun with JNK/SAPK as intermediates. The self-sustaining of this loop may be facilitated by the suppression of the constitutive transcription factor ATF-2, a putative competitor of c-Jun for JNK/SAPK.

Application of the immunosuppressant FK506 shortly after onset of ischemia reduced brain damage and prevented the occurrence of apoptotic nuclei. Also, FK506 prevented the upregulation of DILs, the suppression of ATF-2 and the phosphorylation of c-Jun –but not its expression– in the postischemic brain. In neuroblastoma cells, FK506 counteracted both the doxorubicin-mediated cell death and the subsequent upregulation of DILs and c-jun mRNA. I hypothesize that this drug may neuroprotect either by preventing the calcium-mediated

activation of JNK/SAPK, or by activating the protein kinase C, or by increasing the mitochondrial membrane potential. The exact steps blocked by FK506 remain to be defined. The discovery of the antiapoptotic actions of FK506 following brain ischemia opens new possibilities for the treatment of stroke.