Cytotoxicity of FK506 through via TRAIL, Fas, and TRAIL-4 Signaling Pathway in Human Jurkat T Cells

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Introduction

To elucidate the mechanism of cytotoxicity in FK506-treated Jurkat T cells, signal transduction pathway of TNF-related events was studied. We will further evaluate the
roles of TNF-related death receptors and endoplasmic reticulum-related proteins on the death of Jurkat cells after treatment with FK506.

Methods

Viability of Jurkat T cells was measure by MTT assay.
The catalytic activation of caspase-3 and caspase-9 proteases was determined by digestion of fluorogenic biosubstrates and Western blot with anti-caspase-3 and anti-caspase-9 antibodies. The levels of mRNA and proteins for p53, Bax, PUMA, Proline oxidase, TRAIL(TNF related apoptosis inducing ligand), TRAIL-R1(DR4), TRAIL-R2(DR5), Fas, Fas-L, TNF-α, IL-6, and NK-kB were measured by RT-PCR and Western blot with specific antibodies. Also we further examined the localization of TRAIL family proteins using by fluorescent microscope with specific TRAIL family antibodies.

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Results

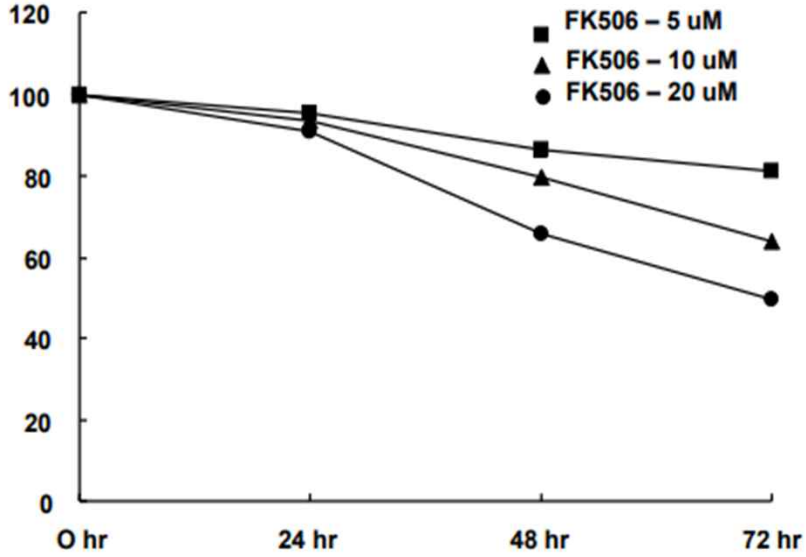
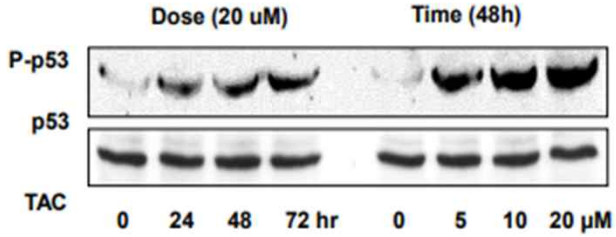


Figure 1. FK506 reduced the viability of Jurkat cells in dose- and time-dependant manners. Cells were treated with various concentrations of FK506 for 92 hrs and then, viability was measured by MTT assay after FK506 treatment.

1. Determination of p53 phosphorylation in FK506-treated Jurkat T cells



2. Translocation of p53 protein in FK506-treated Jurkat T cells

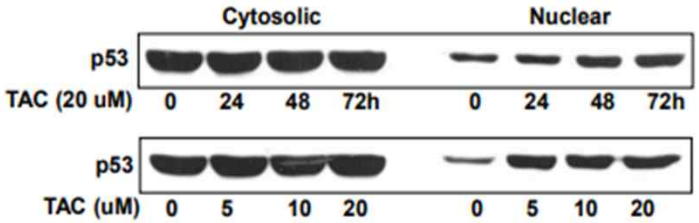
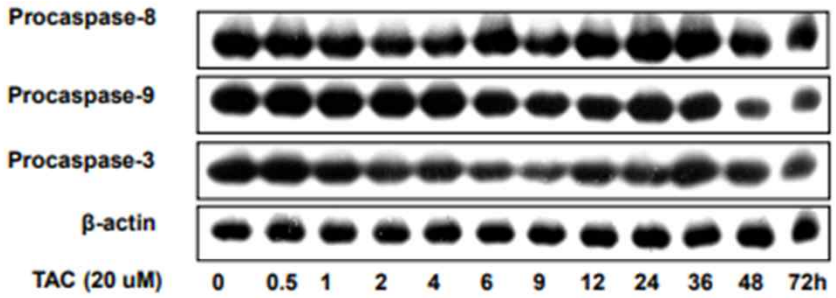


Figure 2. FK506 induces the phosphorylation and nuclear translocation of p53 in Jurkat T cells.

1. Detection of procaspase-3, -8 and -9 by Western blot



2. Detection of active form with caspase-3 and caspase-9

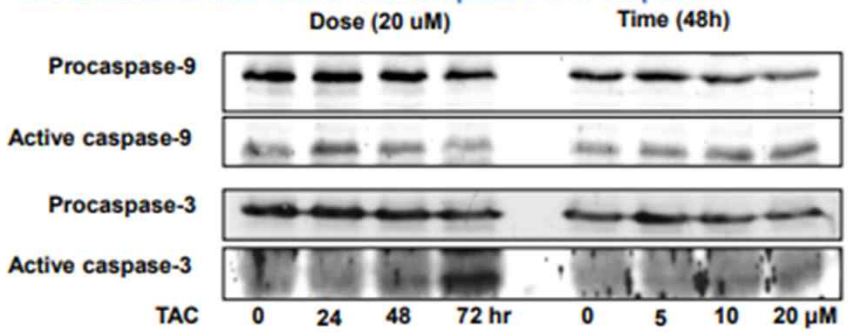
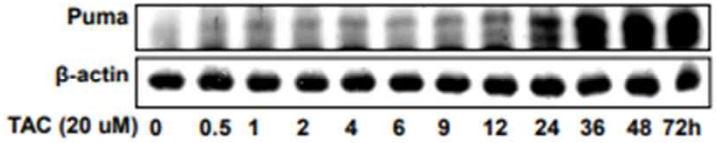
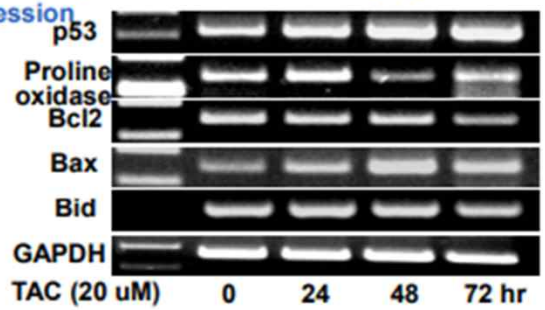


Figure 3. FK506 induces the active form formation of intracellular caspase-3 and caspase-9 in Jurkat T cells.
Jurkat were either present or absent of FK506 20 uM concentration for indicated times and treated with the concentraion of FK506 (5, 10 and 20 uM) for 48 hr.

1. Detection of p53-target genes PUMA induction



2. Detection of p53 and mitochondrial apoptosis related gene expression



3. Detection of the protein expression in mitochondrial membrane stabilization and ROS production

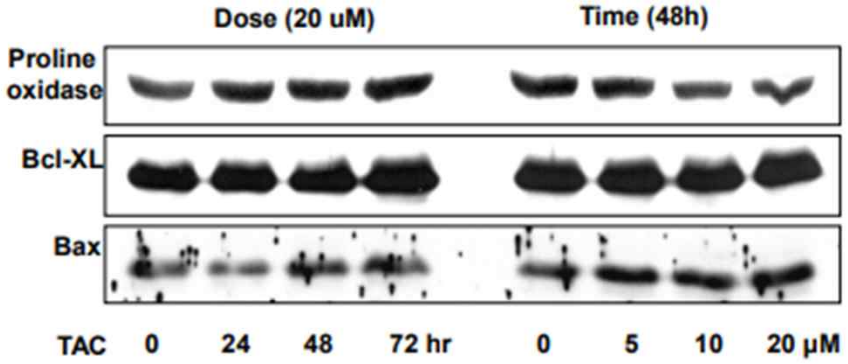


Figure 4. FK506 increase the expression of PUMA, Bax and mitochondrial Proline oxidase via p53 transactivation in Jurkat T cells.
Jurkat were either present or absent of FK506 20 uM concentration for indicated times and treated with the concentraion of FK506 (5, 10 and 20 uM) for 48 hr.

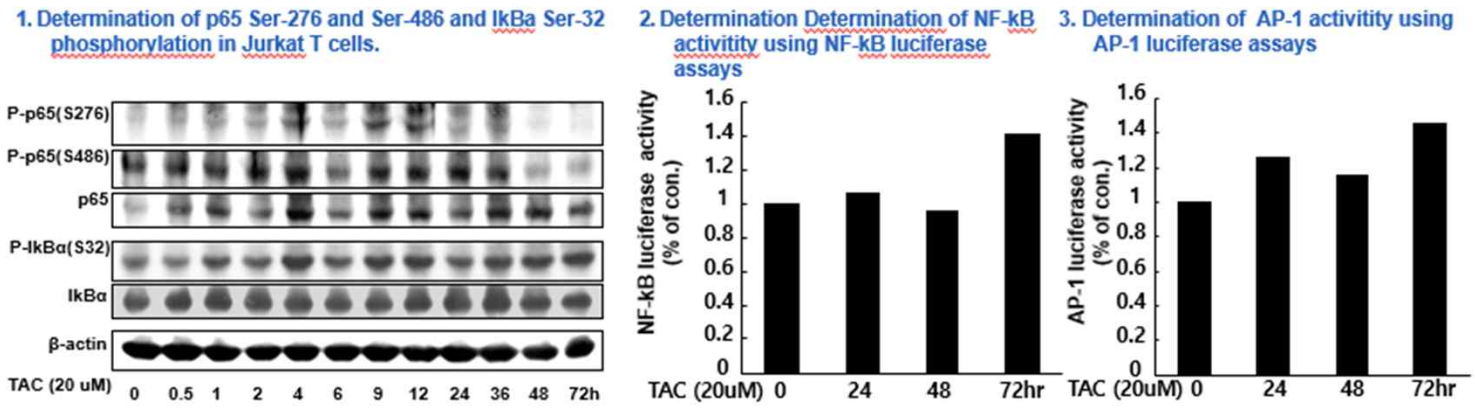


Figure 5. FK506 induces the transactivation of NF-kB through the dephosphorylation of Ser-486 residues in Jurkat T cells.

Jurkat were either present or absent of FK506 20 uM concentration for indicated times and treated with the concentraion of FK506 (5, 10 and 20 uM) for 48 hr. For luciferase assays, Jurkat were transfected with 2 ug/well pNF-kB- and pAP-1-luciferase plasmids and the luciferase activites were measured using a luciferase assay kit (Promega) on a microplate Luminometer.

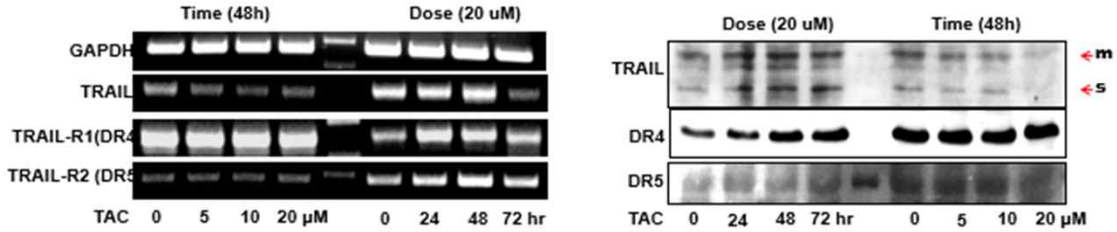


Figure 6. Schematic diagram of FK506-induced death pathway in Jurkat T cells.

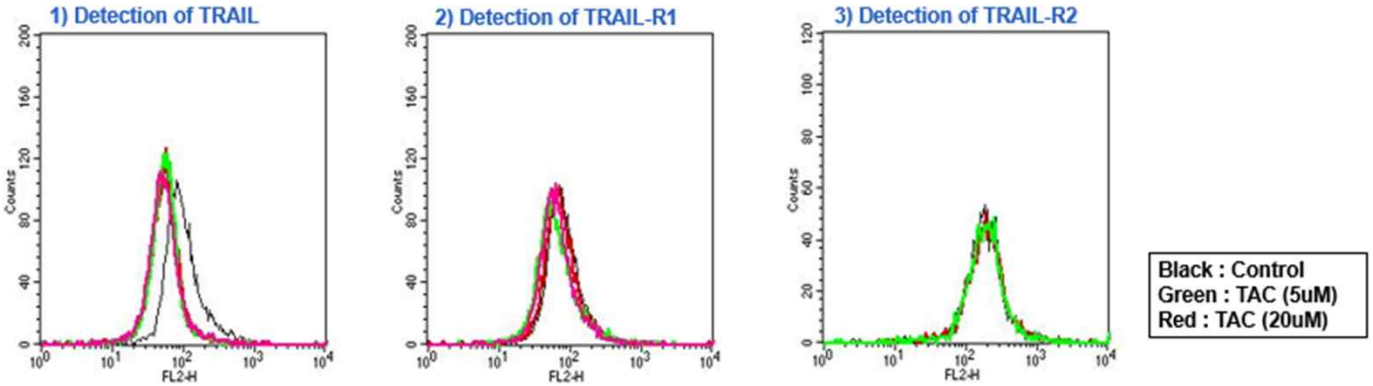
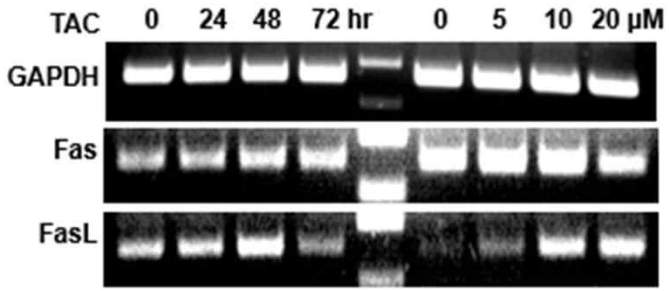


Figure 7. FK506 induces the internalization of TRAIL, TRAIL-R1 and TRAIL-R2 proteins from plasma membrane to cytosol.

1. Determination of FK506-induced Fas and FasL genes mRNA expression by RT-PCR.



2. Detection of FK506-induced Fas and FasL protein expression by Western blot.

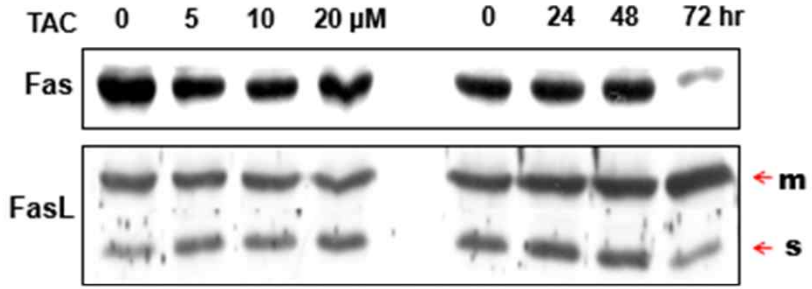
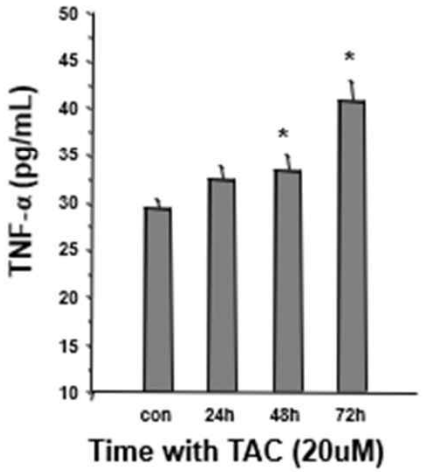
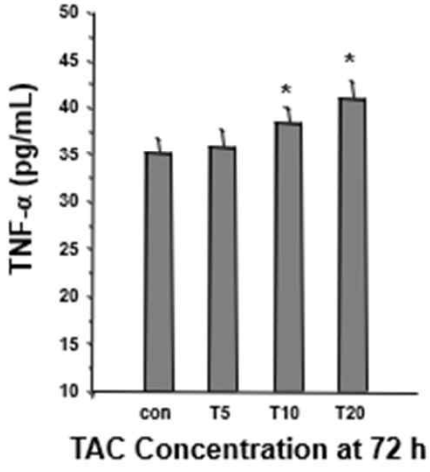


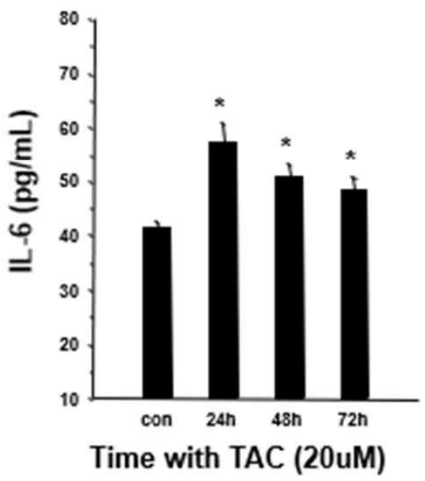
Figure 8. FK506 increased the expressionof Fas and Fas-L genes and proteins.
Jurkat were either present or absent of FK506 20 uM concentration for indicated times and treated with the concentraion of FK506 (5, 10 and 20 uM) for 48 hr.

1. Determination of FK506-induced TNF-α production by ELISA.





2. Determination of FK506-induced IL-6 production by ELISA.



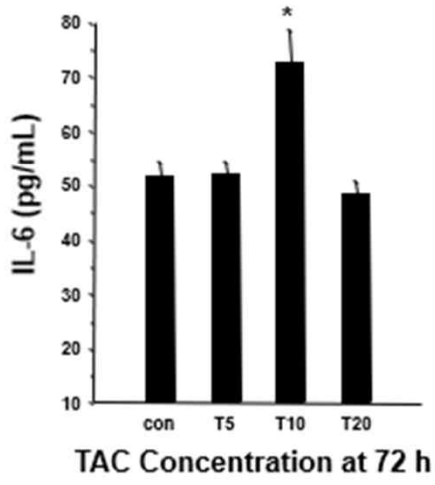
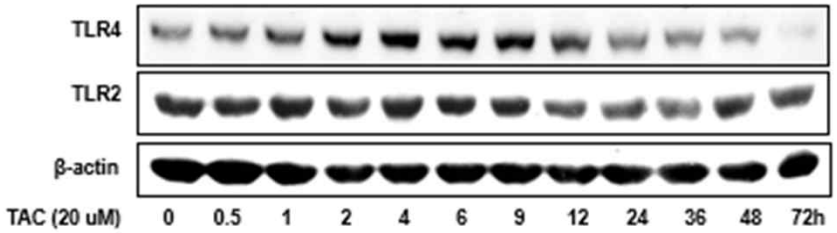


Figure 9. FK506 increased the extracellular release of TNF-α and IL-6 cytokines in Jurkat T cells.

1. Determination of FK506-induced TLR2 and TLR4 production by ELISA.



2. Detection of TLR2 surface expression by FACS analysis.

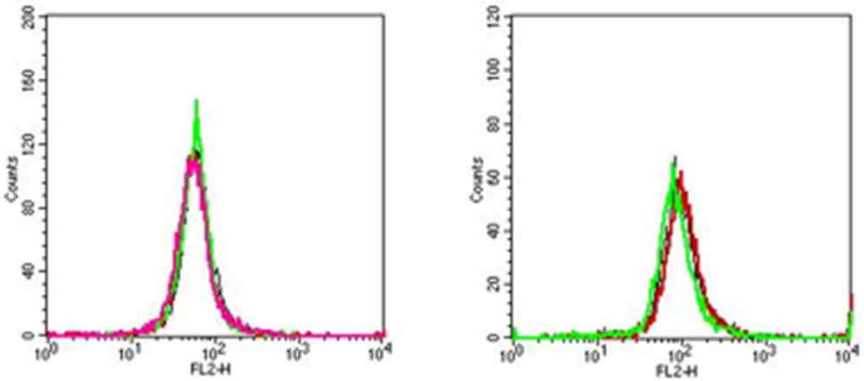


Figure 10. FK506 induces the production of Toll-like receptor TRL4 in Jurkat T cells.

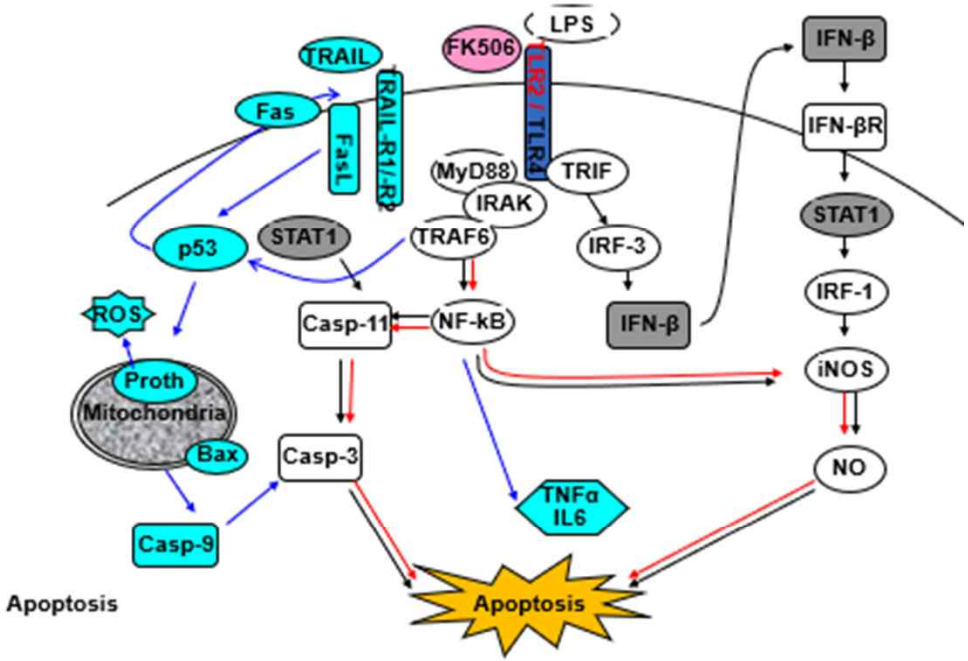


Figure 11. Schematic diagram of FK506-induced death pathway in Jurkat T cells.

Conclusion

These results suggest that FK506 induces apoptotic death of Jurkat cells through activation of caspase family protease, Bcl-2 family protein-related mitochondrial dysfunction, activation of death-receptor and endoplasmic reticulum mediated signaling pathways.