a -Tocopherol as a neutroprotectant: Antioxidant and Non-Antioxidant Mechanisms

Project: 297

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Ziel dieses Projektes war es, den durch Zink verursachten neuronalen Zelltod zu charakterisieren. Für diese Studie wurden Primärzellkulturen aus Kleinhirnneuronen als Modell verwendet um zu erforschen, auf welchem Weg eine Zelle zwischen Nekrose und Apoptose entscheiden kann. Da in der Pathogenese der durch Zink verursachten neurodegenerativen Krankheiten Sauerstoffradikale impliziert wurden, schenkten wir dem schützenden Effekt von ? -Tocopherol besondere Aufmerksamkeit. Wurden Kleinhirnneuronen mit Zink behandelt, wurden sie apoptotisch, nicht aber, wenn sie mit Zink und ? Tocopherol behandelt wurden. ? -Tocopherol war nicht in der Lage, ? -Tocopherol zu ersetzen.

The goal of this project was to characterize key pathways in neuronal cell death resulting from zinc-induced toxicity. The model system employed was primary cultures of cerebellar granule neurons where pathways which lead a cell to decide between necrosis or apoptosis were investigated. Since oxidant events have been implied in the pathogenesis of zinc-promoted neurodegenerative disease, special attention was given to the protective effect of α-tocopherol.
Figure 1 shows cerebellar granule cells (CGC) incubated for 45 min with zinc (10µM) followed by an observation time of 16h in the absence of zinc. Apoptotic neurons are prevalent in panel B (zinc treatment), but not in panels D or F which represent cells treated with zinc plus α- and β-tocopherol (25µM), respectively. Thus CGC in figure 1, and at earlier times, were protected by an antioxidant mechanism.

FIGURE 1

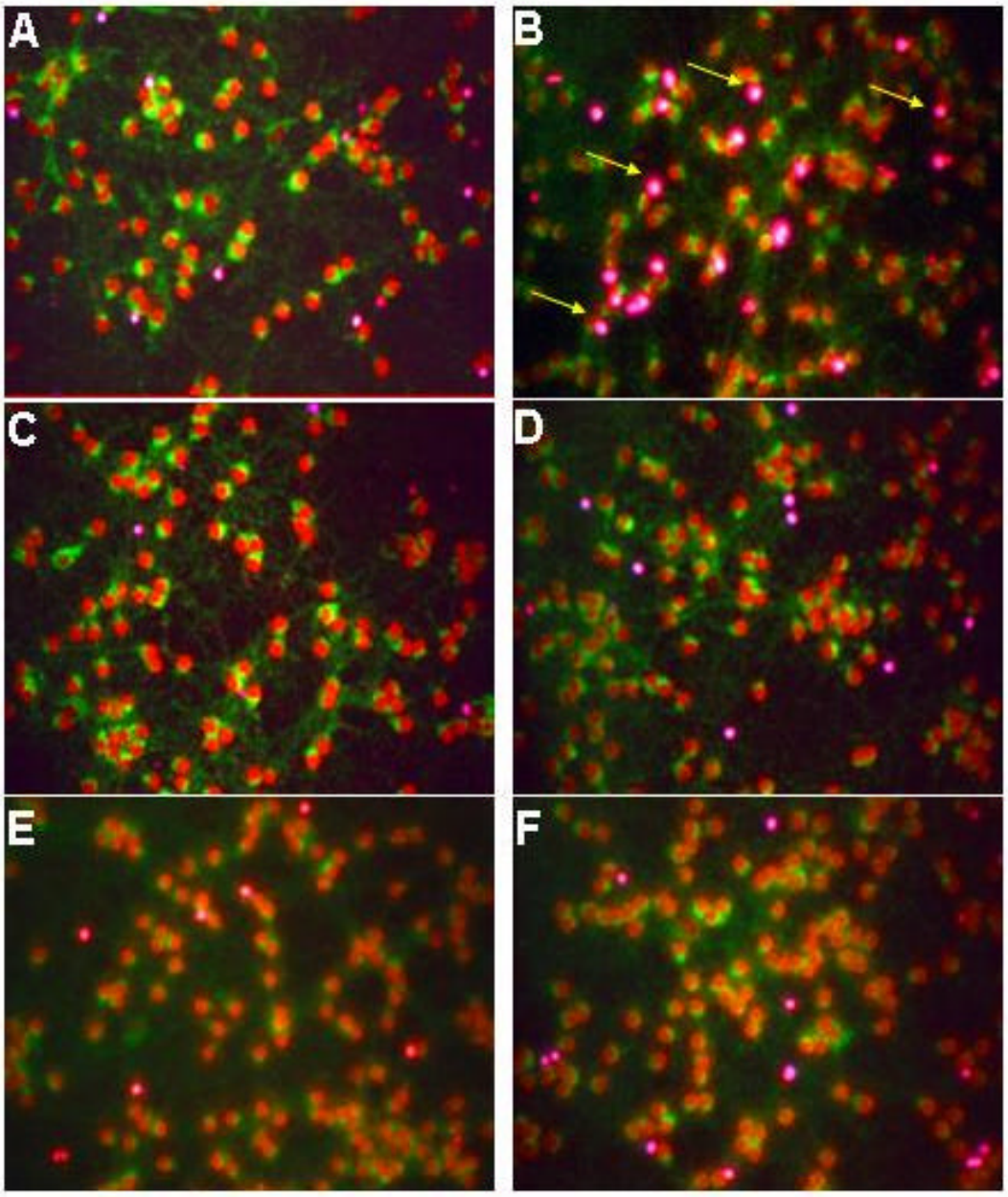


Figure 1. Zinc-induced neuronal apoptosis is equally prevented by α- and β-tocopherol. Cerebellar granule neurons were treated with zinc [10μM] for 45 minutes. Following removal of extracellular zinc via medium exchange, neurons were incubated for 16h, 37°C prior to staining with H-33342 [50ng/ml], SYTOX [500nM], and TMRE [20nM]. Control, α-tocopherol control, and β-tocopherol control (panels A, C, and E, respectively). Zinc treated neurons (panel B), in the presence of α-tocopherol [25μM] (panel D), or treated with β-tocopherol [25μM] (panel F). Examples of apoptotic neurons are indicated with arrows (panel B).

FIGURE 2

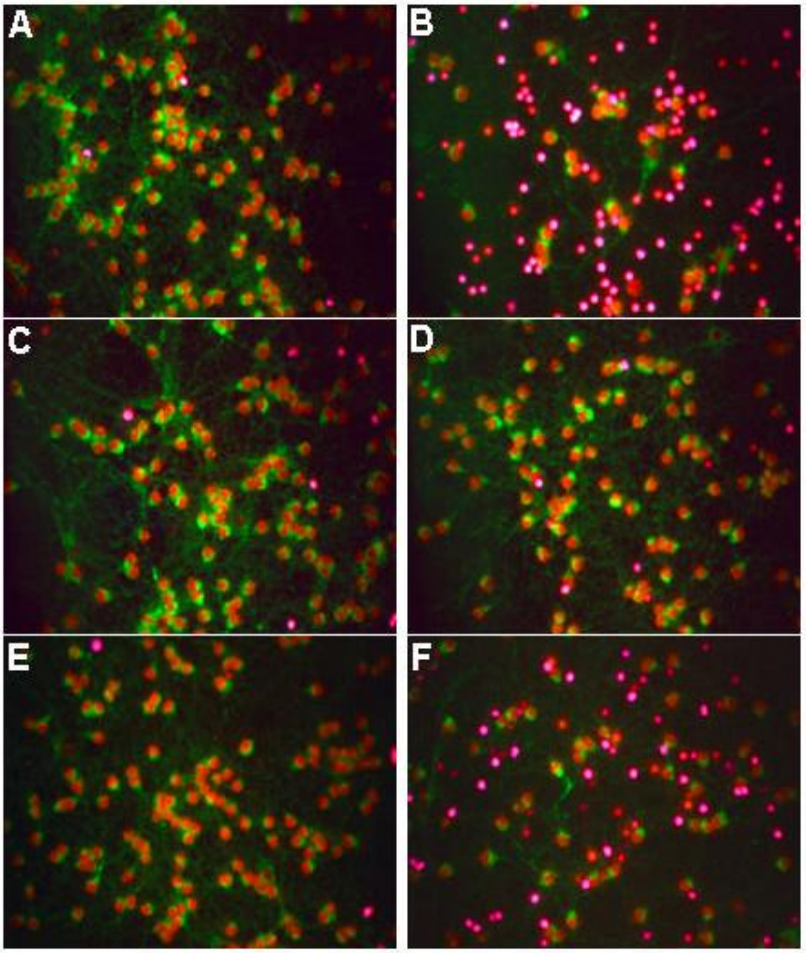
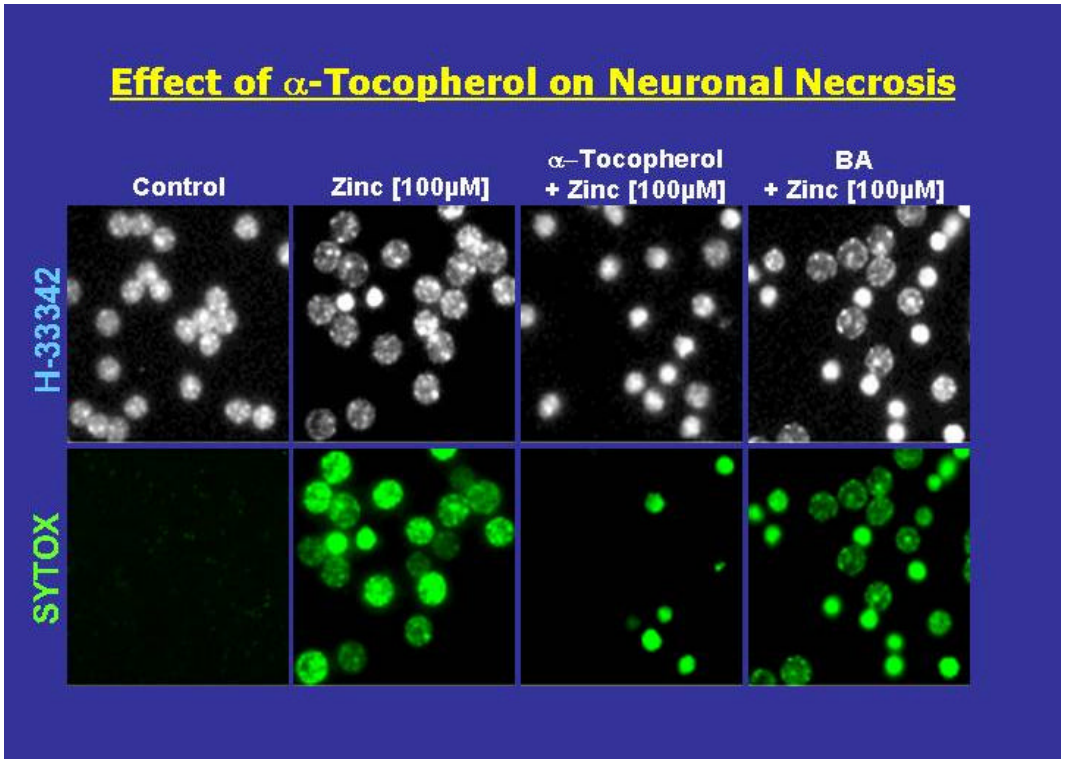


Figure 2. Zinc-induced neuronal apoptosis is prevented by α-tocopherol but not β-tocopherol. Cerebellar granule neurons were treated with zinc [10μM] for 45 minutes. Following removal of extracellular zinc via medium exchange, neurons were incubated for 20h, 37°C prior to staining with H-33342 [50ng/ml], SYTOX [500nM], and TMRE [20nM]. Control, α-tocopherol control, and β-tocopherol control (panels A, C, and E, respectively). Zinc treated neurons (panel B), in the presence of α-tocopherol [25μM] (panel D), or treated with β-tocopherol [25μM] (panel F).

Figure 2 shows dissimilar effects of α- and β-tocopherol. Here sister cultures, following a 20h incubation after zinc treatment (10μM, 45 min), are protected by α-tocopherol (panel D) but not β-tocopherol (panel F). Panel B, represents cells incubated with zinc in the absence of tocopherol, and is statistically indifferent in terms of apoptotic cells from CGC treated with β-tocopherol and zinc (panel F). A conclusion which can be drawn from figure 2 is that the protection afforded by α-tocopherol from zinc-induced neuronal apoptosis is based on a non-antioxidant mechanism of action.

High concentrations of zinc (>50μM) induce neuronal necrosis (Figure 3).



Necrosis is marked here by enlarged, SYTOX (a cell impermeable chromatin stain) positive nuclei. When neurons are exposed to high concentrations of zinc, as is the case in stroke and epilepsy, α-tocopherol did not prevent but rather delayed cell death. Importantly, α-tocopherol changed the shape of cell death from necrosis to apoptosis as evidenced by pycnotic nuclei rather than enlarged SYTOX positive nuclei. The advantage to this is that the inflammatory response associated with necrosis could be avoided thereby minimizing trauma to surrounding neurons.

Bongkrekic acid was employed to investigate the potential involvement of the mitochondrial permeability transition pore (MPTP) in zinc-induced neurotoxicity. As an inhibitor of MPTP, if mitochondrial permeability transition was the mechanism activating a cell death program, we would have expected to see neuroprotection by this substance. The results indicate no protection of neurotoxicity with bongkrekic acid.

Caspase involvement in both models of zinc-induced neurotoxicity was studied by the application of zvad-fmk, a pan-caspase inhibitor. In neither model could this well established caspase inhibitor prevent cell death indicating a caspase independent mechanism despite mitochondrial cytochrome c release. Using another means to evaluate if caspases could play a role in zinc-induced neuronal death the degradation of spectrin was investigated. Spectrin is digested most readily by caspase 3 yielding a 120kDa fragment detectable by western blot. In no experiment was a 120kDa fragment detected, supporting the zvad-fmk results. Caspase-3-like activity was further measured by DEVD-afc cleavage

and assayed spectrophotometrically. Extracts from zinc-treated neurons showed no caspase activity, while extracts from cells treated with staurosporine or colchicine reacted positively.

Zinc induced a dose and time dependent depletion of cellular ATP. Both tocopherols buffered the ATP depletion but could not prevent this phenomenon. Zvad-fmk and bongkrekic acid were totally ineffective in preventing the zinc-mediated neuronal ATP loss.

The project described above yielded important results that allow a better understanding of the mechanisms underlying zinc-mediated neuronal injury. Protection evidenced by tocopherol opens a new area for further research. It would be important to discover if protein kinase C modulation by α-tocopherol is responsible for the long term protection of CGC by α-tocopherol, to determine if this phenomenon is a posttranslational or a transcriptional event, to study if the transcription of certain genes is affected by α-tocopherol in cerebellar granule neurons. The study has not been yet published due to the need of some more experiments to be able to have quantitative data. In the meantime a number of articles have appeared, as a fall-out of the knowledge developed with this study. They are listed below and duly bare acknowledgements to the SFEFS.