

**SYNERGISTIC INTERACTIONS OF NITROXIDES AND
DOXORUBICIN IN THE INDUCTION OF APOPTOSIS: THE PRO-
APOPTOTIC EFFECT OF PIROLIN AND TEMPACE IN
IMMORTALIZED RODENT FIBROBLASTS**

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Anthracycline drugs cause apoptotic death in many types of cells, which is one of the main mechanisms of their cytotoxicity. It has been shown that doxorubicin (DOX) exerts most of its anti-tumor effect through the induction of cell apoptosis. It has been also proposed that the high cardiotoxicity of DOX may result, at least in part, from the induction of cardiac myocyte apoptosis. Low molecular weight nitroxides, non-immunogenic, cell-permeable and non-toxic stable radicals, displaying significant antioxidant activity, have been tested in many studies for their protection against the cardiotoxicity of DOX. Higher doses of these compounds show antiproliferative and toxic effects in some types of cell. The aim of this work was to ascertain whether nitroxides can protect immortalized cells, which we applied as a model for the neoplastic phenotype, against DOX-induced apoptosis. We investigated two structurally different nitroxides: the piperidine nitroxide TEMPACE and the piroline derivative PIROLIN. Apoptosis was estimated via flow cytometry and DNA gel electrophoresis methods. The morphological changes associated with apoptosis were assessed by fluorescence microscopy after cell staining with 33258 Hoechst. We found evidence that both nitroxides alone induce apoptotic changes in cell morphology such as chromatin condensation, nucleus fragmentation and the formation of apoptotic bodies. The pattern of DNA fragmentation observed in TEMPACE/PIROLIN treated cells ("the DNA ladder") was characteristic for the apoptotic mode of cell death. A synergistic interaction of TEMPACE/PIROLIN and DOX was observed in cells pretreated with these nitroxides before incubation with DOX. Both TEMPACE and PIROLIN enhanced the pro-apoptotic effect of DOX resulting in a significant increase in the number of apoptotic cells, suggesting that these nitroxides sensitized immortalized cells to the pro-apoptotic effects of cytotoxic DOX. These results provide insight into another type of the activity of nitroxide compounds – their potential cytotoxic and antiproliferative effect, which should not be overlooked as nitroxide derivatives could be proposed as an useful adjunct treatment of tumors often refractory to standard chemotherapy.