Control of differentiation and cell death of human erythroleukemia cells by alpha1-adrenergic mechanisms

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Background and Aims: Norepinephrine, the major neurotransmitter substance of the sympathetic nervous system plays an important role in the regulation of homeostasis in higher organisms. Few studies have addressed the issue that norepinephrine can also influence erythropoiesis in the bone marrow through adrenergic signaling. The interactions of sympathetic signaling pathways and erythropoiesis can be disturbed by adrenergic stress which is reflected by elevated catecholamine levels in the circulation [1]. Recent experiments in our laboratory showed that the *in vitro* treatment of human erythroleukemia cell lines with α1- adrenergic antagonists results in growth inhibition, the induction of apoptosis and a shift towards megakaryocytic differentiation [2]. To confirm the receptor dependence of these observed effects, erythroleukemia cells were either treated with the α1- adrenergic antagonist prazosin alone or in combination with the α1- adrenergic agonist naphazoline.

Methods: K562 cells were analyzed after α1-adrenergic agonist (naphazoline) and α1-adrenergic antagonist (prazosin) treatment in proliferation and apoptosis assays, FACS analyses and transmission electron microscopy.

Results: Both treatment of human erythroleukemia cells with either the α1-agonist or the α1- antagonist resulted in growth inhibition accompanied by the induction of cell death in a concentration dependent manner. Interestingly, α1- agonist treatment induced an autophagoloytic/necrotic cell death at high drug concentrations (Fig.1), whereas low dose α1-antagonist treatment caused an apoptotic cell death in K562 cells. Aside from growth and viability of the cells, also the morphology and differentiation characteristics of the cells were influenced by both α1- agonist and α1- antagonist treatment. α1- agonist treatment induced aggregation of the cells in culture and suppressed the expression of the erythroid marker glycophorin – a (GPA). On the contrary, α1- antagonist treatment resulted in an increase in cell size, the appeareance of polylobulated nuclei (Fig.1), an increase in DNA content up to 16N (Fig.2) and a weak expression of the megakaryocytic marker CD41a parallel to a loss of glycophorin- a -positive cells. The described effects observed after α1- antagonist treatment are all typical signs of a shift towards megakaryocytic differentiation of K562 cells [2].

When K562 cells were treated with a combination of prazosin and non toxic concentrations of naphazoline, several effects of the antagonist, including growth inhibition, the induction of endomitosis (Fig.2) and also apoptosis, could be attenuated, which confirms the receptor dependence of the observed effects.

Conclusion: We explored a novel α1- adrenergic receptor mediated pathway for the control of both differentiation and survival of human erythroleukemia cells, even though the

relevance of the effect on physiologic erythroid/megakaryocytic progenitor cells must still be examined. The fact that adrenergic antagonist treatment can reversibly induce differentiation as well as apoptosis in leukemia cells could be the basis for further research about the use of adrenergic antagonists in the treatment of leukemia.

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2. R. Fuchs et al., Ann. Hematol. (2009) Epub ahead of print.
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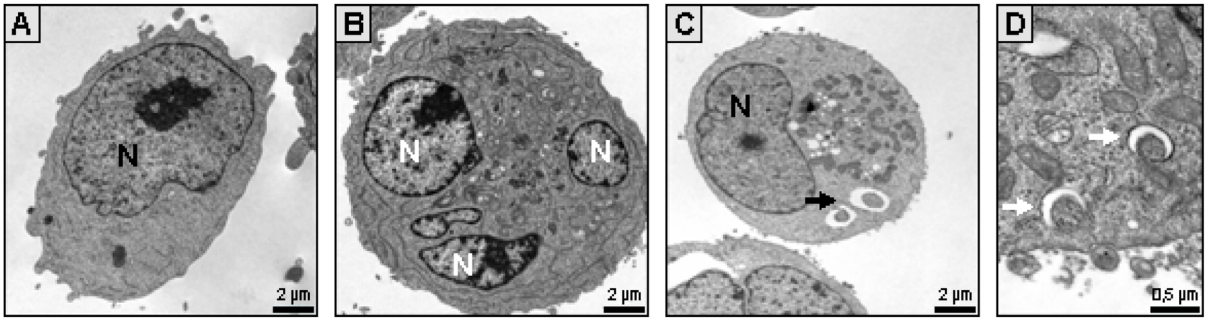

Figure 1. Ultrastructure analysis of naphazoline and prazosin treated K562 cells. After 48 h cultivation of cells without treatment (A) or addition of either prazosin [10 µM] (B) or naphazoline [200 µM] (C, D), cells were analyzed by transmission electron microscopy. Prazosin treated cells exhibited a significant increase in cell size and nuclear complexity (B), whereas naphazoline treated cells displayed an autophagolytic process, characterized by the appeareance of different states auf autophagosomes (C, D). *N*: nucleus, the arrows in figures C and D indicate autophagosomes.

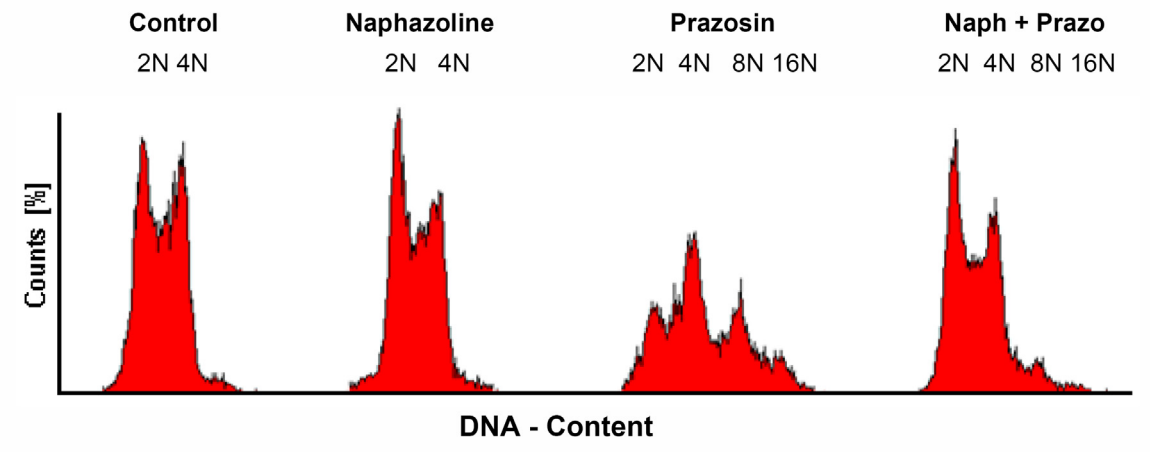

Figure 2. DNA content of the human erythroleukemia cell line K562 after 72 h treatment with the α1-adrenergic agonist naphazoline [100 µM], the α1-adrenergic antagonist prazosin [10 µM] or combinations of both drugs. DNA content of the cells was analyzed by staining with propidiumiodide using a FACScalibur flowcytometer. Prazosin treatment induced an increase in the DNA content of K562 cells up to 16N. The parallel addition of both antagonist and agonist abolished the antagonist's effect on the increase in the DNA content of the cells.

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