Induction of caspase 3 activity, bcl-2 bax and p65 gene expression modulation in human acute promyelocytic leukemia HL-60 cells by doxorubicin with amifostine

Marek Róžalski1, Marek Mirowski1, Ewa Balcerczak1, Urszula Krajewska1, Wojciech Młynarski2, Ryszard Wierzbicki1

1 Department of Pharmaceutical Biochemistry, Molecular Biology Laboratory, 2 Clinic of Pediatrics, Institute of Pediatrics, Medical University of Łódź, Muszyńskiego 1, PL 90-151 Łódź, Poland

Correspondence: Marek Mirowski, e-mail: mirowski@ich.pharm.am.lodz.pl

Abstract:
The influence of amifostine alone and in combination with doxorubicin, cytarabine, and etoposide on the cell growth and on bcl-2, bax and p65 gene expression was investigated in human acute promyelocytic leukemia cell line HL-60. No or very little influence of the exposure of HL-60 cells to amifostine (10^-6 to 10^-5 M) on cell proliferation was shown. Proliferation of HL-60 cells exposed to doxorubicin, cytarabine, or etoposide dropped down with increasing doses of these drugs. Only in the case of doxorubicin, more effective inhibition of HL-60 cell growth was observed when combination of doxorubicin, cytarabine or etoposide with amifostine was used. Cytotoxic effect of cytarabine or etoposide was not reduced by amifostine. The lowering of the cytotoxic index (IC50) was observed only when HL-60 cells were preincubated with amifostine followed by doxorubicin treatment. IC50 was estimated as 2.1 × 10^-7 M and 0.9 × 10^-7 M for doxorubicin and doxorubicin with amifostine, respectively. This effect was accompanied by the induction of caspase 3 activity. HL-60 cells treated with doxorubicin alone showed about 35-fold increase in caspase 3 activity. The enzyme activity was stimulated by combination of doxorubicin with amifostine up to 94 times. Furthermore, the expression of bcl-2 and bax genes involved in apoptosis as well as tumor-associated p65 gene were determined. Semiquantitative reverse transcriptase polymerase chain reaction showed a decrease in bcl-2 and an increase in bax and p65 expression in HL-60 cells treated with doxorubicin in combination with amifostine when compared with the cells treated only with doxorubicin. Amifostine may potentiate doxorubicin therapeutic efficiency in human acute promyelocytic leukemia cells.

Key words:
HL-60 cell line, amifostine, doxorubicin

Introduction

Doxorubicin (adriamycin), cytarabine (1-β-D-arabinosycytosine, ara-C) and etoposide (VP-16) belong to the most commonly used classes of anticancer