Evaluation of chemoprotective effects of amifostine and cysteamine in cell cultures treated with paclitaxel using growth rate parameter

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Abstract: In our study, the cytotoxic effect of paclitaxel (PAC) in tumor and normal cell lines were established, and chemoprotective effects of amifostine (AMI) and cysteamine (CYS) against this effect were examined. Tumor cell lines used in this study were L-strain cells and HeLa cells. Mouse embryonic fibroblasts (MEF) were used as normal cell line. Results of the experiments were evaluated with growth rate parameter. In the experiments, PAC concentrations of 6 and 12 μg/mL were applied to the whole cell lines for 1–10 days, either alone or in combination with 1 μg/mL concentration of AMI and CYS. A statistically significant effect were not determined in the whole cell lines, for the test groups in which 1 μg/mL of AMI and CYS concentrations were applied solely. PAC concentrations caused increasing cytotoxic effect with increasing treatment time ($p < 0.05$). The effect of PAC on repression of cell reproduction appeared to be different in normal and tumor cells. In comparison to tumor cells, MEF cells appeared more resistant to the decreased cell reproduction caused by PAC. HeLa cells were the most sensitive among tumor cells. In the MEF cell line AMI and CYS were cytoprotective. This cytoprotectivity exerted by these agents and the toxicity exerted by PAC alone appeared at the same time period. As a result, PAC continued to show its cytotoxic effect in tumor cells where AMI and CYS were used in combination. In contrast, this effect disappeared in normal cell line. In our study, AMI and CYS did not protect tumor cells, but the protection was observed in normal cells. This study was conducted to understand whether the interaction of the cytoprotective agents AMI and CYS with the chemotherapy agent PAC play role on growth rate. It was also assessed to what extent the cytotoxic and protective effects were agonistic or antagonistic in tumor or normal cells.

Key words: Paclitaxel, amifostine, cysteamine, cytotoxic, chemoprotective, growth rate.

Abbreviations: AMI, amifostine; CYS, cysteamine; MEF, mouse embryonic fibroblast; PAC, paclitaxel.

Introduction

Paclitaxel (PAC) is an antimicrotubule chemotherapy agent. It is a diterpene isolated from the bark of the Western (Pacific) yew, *Taxus brevifolia*. PAC induces the formation of abnormal arrays or bundles of microtubules throughout the cell cycle and of multiple asters of microtubules during mitosis. PAC also inhibits the transition from G0 to S phase by disrupting tubulin in the cell membrane and/or direct inhibition of the cytoskeleton interrupting intracellular transport and communications. This mechanism of action is unlike that of other cytotoxics. The biological effects of PAC, including its observed antitumor activity, are, in all probability, primarily related to the drug's ability to promote the assembly of microtubules from tubulin dimmers and stabilize microtubule by preventing depolymerization. PAC has been used alone or in combination with other cytotoxic agents in the treatment of a variety of animal and human tumor cell lines (Schiff et al., 1979; Rowinsky et al., 1992).

Amifostine (AMI; WR-2721), a phosphorylated thiol, demonstrated the unique ability to protect normal but not tumor tissue from cytotoxic damage induced by radiation therapy and chemotherapy. AMI demonstrates the unique ability to protect selectively a broad range of normal tissues. This selective protection is based on differential dephosphorylation by alkaline phosphatase at tissue site and the preferential uptake of the active thiol metabolite, WR-1065, by cells in normal tissue (Links & Lewis, 1999; Gelmon et al., 1999). The cytoprotective effect of AMI is thought to be a consequence of its ability to scavenge reactive oxygen species and its apoptosis-inducing effect (Davis et al., 2001). Apoptotic response of cells to anticancer drug is mediated by wild-type p53. The role of p53 in AMI effect mechanism is controversial and appears to depend on the cell type (Lee et al., 2003).

In addition to the cytoprotective effects of WR-1065, two additional metabolites, cysteamine (CYS; 2-aminoethanethiol) and the symmetric disulfide WR-33278, have cytoprotective properties. Most of the ex-