Amifostine Aminothiols and Protection of Keratinocyte Apoptosis and DNA Damage

To the Editor:

Amifostine (S-2,3-aminopropylaminoethy phosphorothioic acid), also known as WR-2721, is an aminothiol developed during the 1950s by the U.S. Army as a radio-protective agent (Murray, 1998). Amifostine is dephosphorylated in vivo to the active metabolite WR-1065, which enters cells by passive diffusion (Mitchell et al., 1995) and acts as a potent scavenger of free radicals and inhibitor of DNA damage (Peters and van der Vijgh, 1995). Amifostine protection of human leukemia cells against apoptosis induced by ionizing radiation (IR) relates to its ability to prevent DNA strand breaks (Warters et al., 1997). It effectively protects normal tissues against the acute effects of IR and chemotherapy, and the drug is widely used in cancer patients to increase the therapeutic benefit of radiotherapy and chemotherapy (Wasserman, 1999). Amifostine has been particularly useful in reducing nephrotoxicity associated with cisplatin administration (Markman, 1998) and radiation-induced xerostomia in patients with squamous cell carcinoma of the head and neck (Brizel et al., 2000). In addition, amifostine in some cases has demonstrated anti-proliferative effects (Rubin et al., 1996) and anti-tumor effects (Grima et al., 2002), suggesting its potential as a chemopreventive as well as chemoprotective agent.

To the best of our knowledge, there are no studies examining direct effects of these aminothiols on keratinocytes and whether they can block keratinocyte apoptosis induced by IR. In addition, their potential as protective agents against ultraviolet (UV) B radiation, the primary carcinogen in sunlight and etiologic agent for nonmelanoma skin cancers, has not been formally addressed.

We thus examined the activity of both WR-1065 and amifostine on the model keratinocyte cell line HaCat, which undergoes apoptosis in response to both IR and UVB (Petit-Frere et al., 2000). First, HaCat cells (Grossman et al., 1999a) were preincubated for 15 min in Falcon 353001 dishes in the presence or absence of freshly prepared WR-1065 (kindly provided by Dr Robert Schultz, Drug Synthesis and Chemistry Branch, NCI, Bethesda, MD) or amifostine (Ethylol, Alza Pharmaceuticals, Palo Alto, CA), and then exposed to IR (7.5 Gy per min, J.L. Shepherd 137Cs source) and culturing for 72 h, apoptosis was assessed by propidium iodide staining of cellular DNA and flow cytometry as described previously (Grossman et al., 1999b). As shown in Fig 1(A), IR induced a considerable level of apoptosis that was significantly reduced (p = 0.004, 0.01) in the presence of WR-1065. The amifostine prodrug had a milder protective effect (not shown), likely due to its decreased cell permeability compared with WR-1065 (Calabro-Jones et al., 1985). Concentrations of either drug in excess of 5 mM resulted in variable cytotoxicity, and concentrations of 1 mM were less protective (not shown).

Next, we examined the ability of these aminothiols to protect against UVB radiation. HaCat cells were preincubated in the presence or absence of amifostine and WR1065 and then exposed to UVB as above. Cyclobutane pyrimidine dimers (CPD) and 6-4 pyrimidine-pyrimidone (6-4) photoproducts were detected using TDM-2 and 64M-2 monoclonal antibodies (Mori et al., 1991), respectively (kindly provided by Dr Toshio Mori, Nara Medical University, Nara, Japan). Genomic DNA was prepared as described previously (Grossman et al., 2001) and spotted on Biotrace PVDF membrane (ISC BioExpress, Kaysville, UT) for immunodot-blot assay as described elsewhere (Adinolfi et al., 2001). As shown in Fig 2, both CPD and 6-4 photoproducts were readily detected immediately following higher levels of UVB exposure, whereas none were seen in unirradiated cells. In addition, UV photoproducts were not detected in cells exposed to 10 or 20 Gy IR (not shown). Consistent with the apoptosis responses above, in aminothiol-treated HaCat cells there was no reduction in levels of either UV photoproduct compared with cells not exposed to the drugs at the time points examined (Fig 2).

We have thus shown that the aminothiol amifostine, and particularly its active metabolite WR1065, can protect keratinocytes from apoptosis induced by IR. Reduced keratinocyte apoptosis is the likely basis for the tissue-sparing effect seen in patients with head and neck squamous cell carcinoma receiving amifostine prior to radiotherapy. Our data suggest, however, that these aminothiols are limited in their protective capacity for keratinocytes to IR-induced DNA damage, and thus are likely not to be useful as UV-protective agents in skin.

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Figure 1. Apoptotic responses of HaCat keratinocytes and the effect of WR-1065 preincubation. HaCat cells were either untreated (○) or preincubated (●) with 5 mM WR-1065 for 15 min prior to exposure to the indicated doses of (a) IR or (b) UVB. After 72 h (a) or 24 h (b), all cells were recovered and percent apoptosis was assessed by propidium iodide staining and flow cytometry. Apoptotic cells were identified as the sub-G1 (subdiploid) population and quantitated as a percentage of total cell population. Error bars indicate SEM from four independent experiments performed. p-values determined by unpaired t tests using Prism (Graphpad software, San Diego, CA): *p = 0.004, **p = 0.01. p-values for unirradiated cells and UVB-irradiated cells were not significant (p > 0.05).

REFERENCES


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