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N-Acetylcysteine interacts with copper to generate hydrogen peroxide and selectively induce cancer cell death

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ABSTRACT

A variety of metal-binding compounds have been found to exert anti-cancer activity. We postulated that N-acetylcysteine (NAC), which is a membrane-permeable metal-binding compound, might have anti-cancer activity in the presence of metals. We found that NAC/Cu(II) significantly alters growth and induces apoptosis in human cancer lines, yet NAC/Zn(II) and NAC/Fe(III) do not. We further confirmed that this cytotoxicity of NAC/Cu(II) is attributed to reactive oxygen species (ROS). These findings indicate that the combination of Cu(II) and thiols generates cytotoxic ROS that induce apoptosis in cancer cells. They also indicate a fourth class of anti-neoplastic metal-binding compounds, the "ROS generator".

1. Introduction

Some metal-binding compounds exhibit anti-cancer activity, due to three distinct mechanisms of action [1–3]. The most well-recognized is "chelation" (also called "sequestration"), whereby cells are killed because they are functionally deprived of a required metal [3,4]. The effects of a chelator are reversed by supplying the sequestered metal. Other metal-binding compounds kill cancer cells by transporting metals into cells, triggering apoptotic pathways [5–8]. Some act as "ionophores" and others as "shuttles". An ionophore is a compound capable of sequentially transporting multiple metal ions into cells. A compound killing cells by acting as an ionophore can be recognized functionally by showing that the toxicity of a fixed concentration of the ionophore is potentiated by the addition of increasing concentrations of the effector metal. For example, the cytotoxicity of a zinc ionophore (clioquinol), is potentiated by increasing the concentration of zinc, while the toxicity of a zinc chelator (TPEN) is reduced by additional zinc [4]. Shuttles compose a third group of cytotoxic metal-binding compounds. By binding the metal, they facilitate the transport of the metal into the cell. Shuttles differ from ionophores in that their cytotoxic activity is not potentiated by adding excess metal [9,10]. For reasons yet to be fully elucidated, cancer cells are more susceptible to a number of metal-binding compounds than are non-malignant cells.

Abbreviations: BCS, bathocuproinedisulfonic acid; NAC, N-acetylcysteine; MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); PARP, poly (ADP-ribose) polymerase; ROS, reactive oxygen species; SOD, superoxide dismutase; TM, tetrathiomolybdate; TPEN, (N,N,N′N′-tetrakis(−)[2-pyridylmethyl]-ethylenediamine.

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We postulated that an additional number of known metal-binding compounds might have unrecognized anti-cancer activity, by serving as chelators, ionophores, or shuttles. Active compounds would be expected to have both a metal-binding domain as well as domains capable of mediating passage across the plasma membrane. Identification of compounds with these properties and a record of safe human administration would provide new potential anti-cancer agents.

One well known metal-binding domain is the thiol (–SH) group, which is found in many small molecules, as well as proteins. We thus considered whether thiol-containing compounds that have been approved for clinical use have unrecognized anti-cancer properties, and began by examining N-acetylcysteine (NAC). NAC has been used for many years as an antioxidant in biomedical research as well as in clinical practice [11,12]. Glutathione is the major antioxidant of the cytoplasm and its synthesis can be limited *in vivo* by the availability of cysteine [13–15]. NAC is de-acetylated to cysteine on the cell surface or inside of the cell [16], thereby promoting glutathione formation when intracellular cysteine is limiting and enhancing the antioxidant activity of the cytoplasm. It is known that under certain circumstances, anti-oxidants may also serve as pro-oxidants, and it is not surprising that NAC has also been reported to promote DNA damage, suggestively via an increase in reactive oxygen species [17–19].

Given its chemical and biological profile, we hypothesized that NAC serves as either a metal ionophore or shuttle. We assayed its effect on cell viability in the presence or absence of metals. Unexpectedly, we found that NAC is cytotoxic only when administered with copper, and exerts its effects by a previously unreported mechanism.

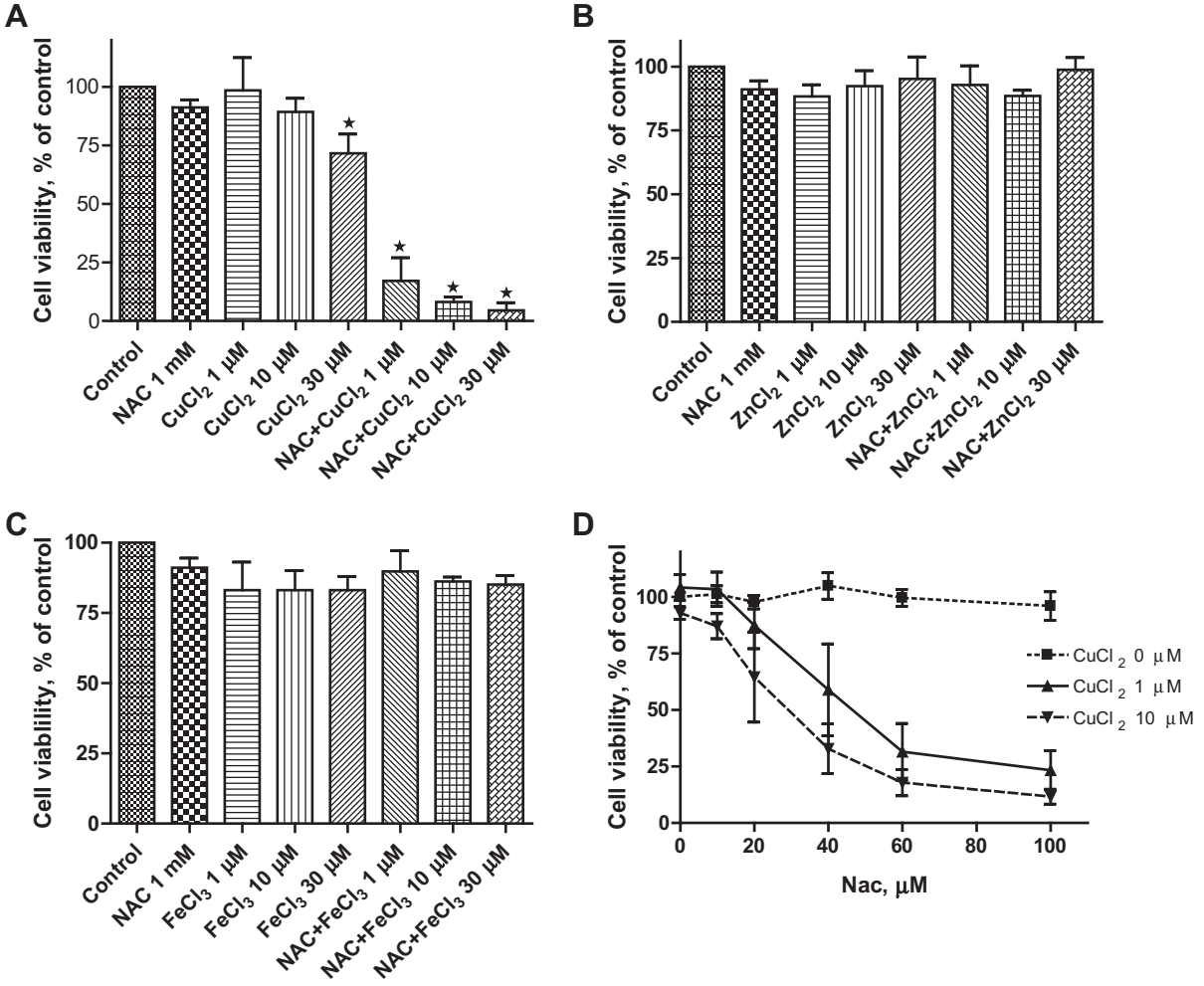

Fig. 1. NAC specifically complexes with copper (Cu), but not zinc (Zn) or iron (Fe), to inhibit cell viability of the A2780 cells. A2780 cells were cultured in RPMI 1640 medium and treated with 0, 1, 10, and 30 μM of $CuCl_2$ (A), $ZnCl_2$ (B), or $FeCl_3$ (C) in the absence or presence of NAC (1 mM) for 72 h and cell viability was examined by the MTS assay. (D) A2780 cells were treated with increasing concentrations of NAC ranging from 0 to 100 μM in the presence of $CuCl_2$ (1 or 10 μM) for 72 h and cell viability was examined by the MTS assay. Data (means ± SD, $n = 3$) are expressed as percentages of the MTS level detected in untreated control cells.