The cell death response to γ-radiation in MCF-7 cells is enhanced by a neuroleptic drug, pimozide

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Summary

Neuroleptic drugs that bind sigma sites were tested for their ability to inhibit growth and radiosensitize MCF-7 human breast cancer cells. Inhibition of growth by ~ 50% occurred in cells exposed to pimozide (0.6 μM), haloperidol (10 μM), and the sigma ligand DTG (1,3-di(2-toly)guanidine, 20 μM), but no growth inhibition occurred in cells exposed to clozapine, a neuroleptic drug lacking sigma binding activity, or dextromethorphan, a selective sigma 1 binding ligand. Pimozide (2.5 μM), but not haloperidol (3.6 μM), enhanced the sensitivity of MCF-7 cells to γ radiation in clonogenic survival assays. Pimozide significantly decreased MCF-7 clonogenic survival following a 5 or 8 Gy dose of γ radiation, and the dose of radiation required for 1% survival (survival enhancement ratio, SFR) was decreased by a factor of 2. Exposure of normal WI-38 human embryonic lung cells to pimozide did not increase their sensitivity to γ radiation. Pimozide (2.5 μM) activated early apoptotic changes in MCF-7 cells that were detected by the uptake of Hoechst 33342 dye, and 10 μM pimozide activated a complete apoptotic pathway resulting in the death of > 90% of the cells within 24 hours. MCF-7 cells exposed to γ radiation alone (8 Gy) showed giant cell formation, mitotic arrest, and a limited degree of apoptosis and necrosis. Within 50 hours of treatment with a combination of radiation and pimozide, cell numbers were sharply reduced compared with cultures exposed to either radiation or pimozide alone. We conclude that pimozide augmented the sensitivity of MCF-7 cells to radiation-induced cell killing through a mechanism not shared by haloperidol, but suggest that concentration of pimozide in MCF-7 cells as a result of an enrichment of sigma 2 sites might target the radiosensitization.

Introduction

Sigma ligands possess the ability to alter cell signaling pathways, but the specific physiological functions of sigma binding sites and the nature of their endogenous ligands, are as yet ill-defined. High affinity sigma 1 receptors are associated with the endoplasmic reticulum membrane [1, 2], and are structurally related to steroid isomerase enzymes. Sensitization of neuronal cells to NMDA, inhibition of cholinergic-induced phosphatidylinositol turnover, and interaction with trimeric G proteins by micromolar concentrations sigma 1 ligands have been reported [3–6]. Sigma 2 sites are hypothesized to play a neuromodulatory role in the brain because in neuronal cells, sigma ligands alter potassium channel activity [7, 8] and responsiveness to NMDA [5]. Sigma ligands have also been shown to alter protein

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phosphorylation and intracellular calcium levels [9].

Neuroleptic drugs have high affinity for sigma 1 and sigma 2 sites [3, 5, 6, 10, 11], and the binding of neuroleptics to sigma sites in the brain has been proposed to play a role in their antipsychotic properties and drug-induced motor disorders [10, 12]. Levels of pimozide have been measured in psychiatric patients and normal volunteers [13]. Following the administration of usual therapeutic doses of pimozide, blood levels of pimozide ranged between 1–20 ng/ml (2–40 nM). Sigma binding sites also occur in peripheral tissues including endocrine organs [14], cells of the immune system [15], blood vessels and other smooth muscle tissues, liver, kidney, spleen, and heart, where their role is less clearly understood [6, 16]. Our interest in sigma sites is based upon the observations that human brain, kidney and colon tumors, as well as cell lines derived from melanomas, breast tumors, and lung carcinomas overexpress sigma 1 sites and/or sigma 2 sites compared with their normal counterparts [17–21]. Cells exposed to high concentrations of neuroleptic drugs (100 μM) in vitro showed morphological characteristics of apoptosis [22]. It was suggested that the cytotoxicity of neuroleptic drugs was related to their ability to bind sigma sites [22], and growth inhibition in human breast carcinoma cells by the neuroleptics pimozide and thioridazine [23, 24], by sigma ligands in a variety of transformed cell lines of neuronal origin [25], and in human mammary adenocarcinoma, colon carcinoma, and melanoma by sigma ligands, particularly rimcazole and reduced haloperidol [26, 27], appeared to support this hypothesis. The disparity between the affinity constants of neuroleptic drugs for sigma sites measured in isolated membranes (Kd 0.2–150 nM) [28–30] and drug concentrations that induce cytotoxicity, 2.5–100 μM [22–27], suggest that the efficacy of sigma ligands in cytotoxicity is low and/or that lower affinity sigma receptors are involved in the cytotoxic responses. Among different sigma ligands there is a good rank order correlation between sigma receptor Kd and the ability to inhibit intracellular signalling pathways at μM concentrations [3, 5].

Mach et al. [3] showed that in a murine mammary adenocarcinoma cell line, high affinity sigma 2 binding sites were preferentially expressed during the proliferation phase of growth compared with quiescent, G1-arrested cells. MCF-7 human mammary adenocarcinoma cells express high affinity sigma 2, but not sigma 1 binding sites [21], and provide an in vitro model for investigating the role of sigma 2 sites in human mammary adenocarcinoma. Based upon the in vivo evidence that sigma ligands concentrate in tumors sufficiently to allow tumor radioimaging [32–34], we tested whether either of the structurally distinct sigma ligands, haloperidol and pimozide, sensitized MCF-7 breast cancer cells to the killing effects of γ-radiation. We found that pimozide, a diphenylbutylpiperidine, but not haloperidol, a butyrophenone, acted as a radiosensitizer in MCF-7 cells in vitro. In addition, we found that pimozide, but not haloperidol, activated programmed cell death in MCF-7 cells.

Materials and methods

Cell culture

A permanent cell line derived from a human breast carcinoma, MCF-7, between passage numbers 40 and 65, was used for these experiments [25]. The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Bio Whittaker, Walkersville, MD) supplemented with 10% fetal bovine serum (FBS) (HyClone Laboratories, Inc., Logan, Utah), 2 mM glutamine, 0.04 mg/ml gentamicin in a 5% CO2-95% air incubator at 37 °C, and passaged weekly at a 1:5 ratio. The WI-38 cell line, passage 6, was obtained from the American Type Culture Collection. WI-38 cells were maintained in Eagle's Minimal Essential Medium (Bio Whittaker) – 10% FBS – 2 mM glutamine and 0.04 mg/ml gentamicin. Cell counts were performed in the presence of 0.02% trypan blue to assess viability using a hemacytometer.

Colony formation assay

Cells (1 × 10^6) were seeded into 35 mm² dishes in DMEM + 5% FBS. Drug or solvent as a control