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SHORT COMMUNICATION

Differential in radiosensitizing potency of enantiomers of the fatty acid synthase inhibitor C75

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Abstract

The elevated activity of fatty acid synthase has been reported in a number of cancer types. Inhibition of this enzyme has been demonstrated to induce cancer cell death and reduce tumor growth. In addition, the fatty acid synthase inhibitor drug C75 has been reported to synergistically enhance the cancer-killing ability of ionizing radiation. However, clinical use of C75 has been limited due to its producing weight loss, believed to be caused by alterations in the activity of carnitine palmitoyltransferase-1. C75 is administered in the form of a racemic mixture of (−) and (+) enantiomers that may differ in their regulation of fatty acid synthase and carnitine palmitoyltransferase-1. Therefore, we assessed the relative cancer-killing potency of different enantiomeric forms of C75 in prostate cancer cells. These results suggest that (−)-C75 is the more cytotoxic enantiomer and has greater radiosensitizing capacity than (+)-C75. These observations will stimulate the development of fatty acid synthase inhibitors that are selective for cancer cells and enhance the tumor-killing activity of ionizing radiation, while minimizing weight loss in cancer patients.

KEYWORDS

C75, fatty acid synthase, ionizing radiation

1 | INTRODUCTION

Fatty acid synthase (FASN) is the enzyme responsible for endogenous synthesis of saturated long-chain fatty acids from the precursors acetyl-CoA and malonyl-CoA. Many common human cancers, including carcinoma of the prostate and breast, express high levels of FASN compared with normal human tissues.[1–3] This elevated expression is associated with protection against apoptosis, increased metastasis, and poor prognosis. Inhibitors of FASN decrease prostate cancer cell proliferation, increase apoptosis, and decrease tumor growth in experimental models. The natural fungal product cerulenin is an inhibitor of FASN and a US Food and Drug Administration (FDA)-approved antiobesity drug. Synthetic derivatives such as the competitive irreversible FASN inhibitor C75 are more chemically stable.

Inhibition of FASN by C75 induced apoptosis in cancer cells. This was probably caused by elevated levels of the FASN substrate malonyl-CoA.[4] Malonyl-CoA also inhibits carnitine palmitoyltransferase (CPT-1), the rate-limiting enzyme of fatty acid oxidation, preventing the oxidation of newly synthesized fatty acids.[5] CPT-1 stimulation by C75, however, does not appear to play a role in the cytotoxic response.[5]

We have previously demonstrated that C75 was cytotoxic to prostate cancer cell lines (PC3 and LNCaP), decreased

survival of PC3 clonogens, and inhibited the growth of multicellular spheroids composed of LNCaP cells.[6] Crucially, C75 enhanced the clonogenic kill and spheroid growth delay induced by experimental radiotherapy in a synergistic manner characteristic of a radiosensitizer.

Although C75 exhibits selective killing of cancer cells, its side effects of anorexia and weight loss have limited its potential for the treatment of cancer patients. C75-induced modulation of CPT-1 appears to be responsible for its capacity to reduce weight. However, the mechanism of action is unresolved. C75 directly activates CPT-1, leading to increased fatty acid β-oxidation with consequent weight loss.[7,8] Conversely, experiments in vitro and in rodent models demonstrated that C75 is converted to C75-CoA.[9,10] Accumulation of C75-CoA and malonyl-CoA, caused by reduced FASN activity, act as signals of nutrient abundance to hypothalamic neurons, inhibiting CPT-1 and mediating the suppression of food intake and further contributing to weight loss.[11] This has led to the evaluation of drugs that inhibit FASN but do not affect CPT-1 activity.[12,13]

Mims et al.[14] recently demonstrated that inhibition of FASN sensitized cancer cells to experimental radiation therapy. However, that study used orlistat, which is poorly soluble and has an extremely low oral bioavailability,[15] thus limiting its clinical application. Radiosensitization of cancer cells was also achievable using C75.[6] However, it is possible that the racemic mixture of C75 may modulate the activity of CPT-1 activity, which is responsible for its undesirable effects on body weight and appetite in patients with cancer.[11] Here we evaluated the radiosensitizing activity of enantiomers of C75 in comparison with the commonly used racemic mixture of C75.

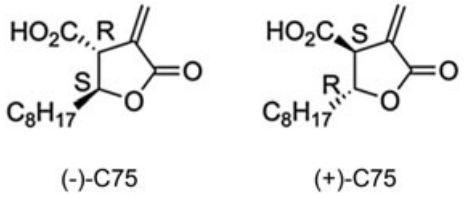

FIGURE 1 Structure of the enantiomers of C75.

2 | MATERIALS AND METHODS

Human prostate cancer cell lines PC3 and LNCaP were obtained from the American Type Culture Collection (Manassas, VA) and were used in this study for less than 6 months after resuscitation. Cell lines were maintained as previously described.[6] Racemic mixtures of C75 were purchased from Sigma-Aldrich (Dorset, UK) and Santa Cruz Biotechnology (Santa Cruz, CA). Enantiomers of C75 [(−)-trans-C75 and (+)-trans-C75] were purchased from Santa Cruz Biotechnology. The structure of the enantiomers of C75 is shown in Figure 1. A methyl tetrazolium (MTT) cytotoxicity assay was performed on cell lines in 96-well plates after administration of drugs for 24 h. For clonogenic assay, PC3 cells in exponential growth phase were treated with drugs alone or in simultaneous combination with radiation using an X-Strahl RS225 X-ray irradiator at a dose rate of 1.6 Gy per min. After 24 h treatment, cells were seeded for clonogenic survival assay. Cells were incubated at 37°C in 5% CO_2 for 10 days. Colonies were fixed in methanol, stained with crystal violet solution, and colonies of at least 50 cells were counted. Data are presented as means ± standard error of the mean (SEM) of three separate experiments. Statistical significance was determined using

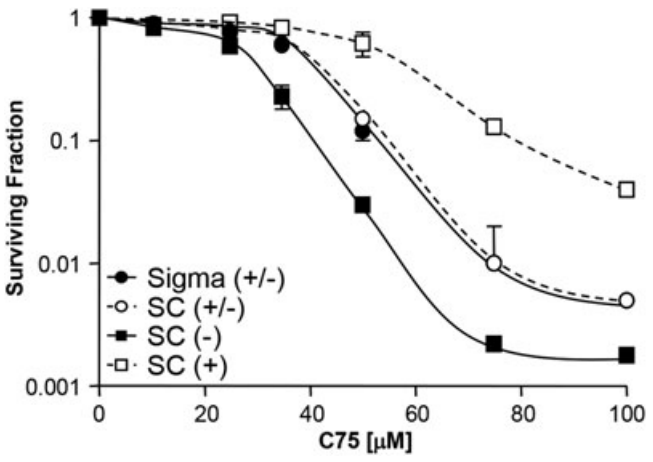

FIGURE 3 Clonogenic survival of PC3 prostate cancer cells in response to racemic mixtures and enantiomers of C75.

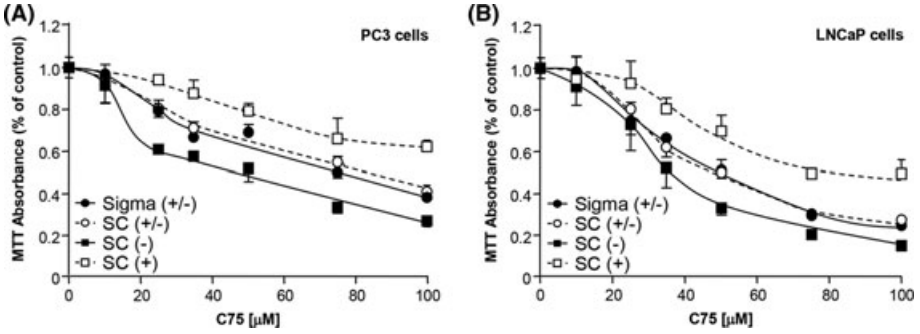

FIGURE 2 Cytotoxicity of different stereoisomers of C75. MTT assay of **A**, PC3 and **B**, LNCaP cells in response to racemic mixtures of C75 (+/−) from two commercial sources (Sigma-Aldrich and Santa Cruz Biotechnology), or (−) and (+) enantiomers of C75 from Santa Cruz Biotechnology.

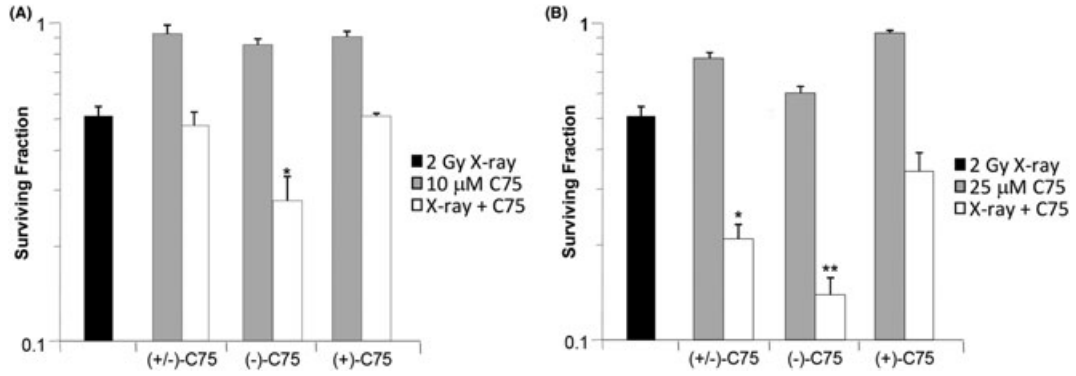

FIGURE 4 Enhancement of the clonogenic killing activity of x-radiation by C75 administered in the form of a racemic mixture (+/−) or (−) and (+) enantiomers (Santa Cruz Biotechnology). PC3 cells were treated simultaneously with x-radiation (2 Gy) and **A**, 10 μM or **B**, 25 μM C75. *$P < 0.05$, **$P < 0.01$ compared with x-radiation alone.

Student's *t*-test. $P < 0.05$ was considered statistically significant and <0.01 highly significant.

3 | RESULTS AND DISCUSSION

The potency of C75, obtained from Sigma-Aldrich and used in previous studies, was compared with a racemic mixture and enantiomers purchased from Santa Cruz Biotechnology. The two racemic mixtures had almost identical cytotoxic potency in both cell lines (Figure 2). However, the (−) enantiomer was more potent than the racemic mixture, whereas the (+) enantiomer had less potency than the racemic mixture in both cell lines. This is in agreement with the antitumor effect of C75 previously described by Makowski et al.[11] in breast and ovarian cancer cell lines, wherein the (+) enantiomer of C75 was less potent than the racemic mixture or the (−) enantiomer. Similar to the cytotoxicity assays, in clonogenic assays of PC3 cells the racemic mixtures had near identical activity, which was less than the (−) enantiomer and more than the (+) enantiomer (Figure 3). In clonogenic assays, the 50% clonogenic kill (IC_{50}) values were 37.01 ± 1.45 and 37.81 ± 1.23 μM for Sigma-Aldrich and Santa Cruz C75, respectively. The (−) enantiomer of C75 had significantly greater clonogenic killing potency (IC_{50} 28.08 ± 0.66 μM) than the racemic mixture, whereas the (+) enantiomer of C75 had substantially less effect, reflected in an IC_{50} value of 51.36 ± 7.67 μM.

For the first time, the radiosensitizing activity of C75 enantiomers was also evaluated. Greater than additive enhancement of radiation-induced clonogenic kill was observed with the racemic mixture of C75 or the (−) enantiomer when administered at 25 μM (Figure 4). In contrast, (+)-C75 did not induce a significant enhancement of radiation-induced clonogenic kill. Crucially, the radiosensitizing activity of (−)-C75 was evident even when administered at 10 μM—a concentration that does not induce significant clonogenic cell kill (Figure 2a).

It has previously been reported that, although C75 had a minimal effect on CPT-1 activity, (+)-C75-CoA inhibited CPT-1 to a significantly greater extent than either racemic C75-CoA or (−)-C75-CoA.[11] Furthermore, when injected into rats the anorectic effect of C75 was largely attributed to the presence of (+)-C75.[11] These findings, combined with the observations that (−)-C75 was able to inhibit FASN activity and was cytotoxic to cancer cell lines, suggested that (−)-C75 is likely to display the beneficial antitumor effects in the absence of significant side effects associated with the racemic C75. Therefore, we assessed the radiopotentiation of the stereoisomers of C75 in vitro and observed that the radiosensitizing activity was a property only of the (−)-C75 enantiomer. Importantly, the clonogenic killing potency of (−)-C75 was greater than either racemic C75 or (+)-C75. It is expected that clinical application of the (−) enantiomer of C75 will improve the treatment of metastatic prostatic carcinoma without inducing adverse anorexia. This combination of (−)-C75 with radiotherapy is also likely to be effective in other tumor types that overexpress fatty acid synthase.

4 | CONCLUSION

We demonstrate here that the antitumor potential and radiosensitizing activity of C75 is due to the (−)-C75 enantiomer. It is expected that therapeutic use of this enantiomer will reduce the previously limiting clinical effect of weight loss in cancer patients.

ACKNOWLEDGMENTS

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