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Research Article

[6]-Gingerol Decreases Clonogenicity And Radioresistance of Human Prostate Cancer Cells

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ABSTRACT

The phenolic compound [6]-Gingerol, isolated from *Zingiber officinale*, has been demonstrated to have antitumor activity for different types of malignant tumours. Prostate cancer is the most common malignancy among males worldwide, being the second leading cause of cancer death in men. In the present study, we investigated the antitumor action of [6]-Gingerol on a human prostate cancer cell line (LNCaP). Our data shows that [6]-Gingerol treatment induced a dose-dependent decrease in the cell viability. Compared with the vehicle control, the cell viabilities were $79.90 \pm 3.56\%$ and $53.06 \pm 7.82\%$ when the LNCaP cells were exposed to 150 $\mu\text{g/mL}$ and 300 $\mu\text{g/mL}$ of [6]-Gingerol, respectively. The treatment of LNCaP with 300 μM of [6]-Gingerol led to a significant reduction (~25%) on the clonogenic survival of these cells. Furthermore, [6]-gingerol acted as a radiosensitizer for LNCaP cells. The pretreatment of these cells with [6]-Gingerol significantly enhanced the killing effects of ionizing radiation with a dose enhancement ratio of 1.25. Our results demonstrate the anti-tumour activities of [6]-Gingerol. Further studies are needed to elucidate the mechanisms involved.

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Introduction

Prostate cancer (PCa) is the second most prevalent malignancy and second leading cause of cancer-related deaths among men in the world [1]. The choice of treatment modality depends on the stage of the disease and the patient's clinical conditions [2]. Radical prostatectomy combined with radiotherapy (RT) is standard treatment for clinically localized PCa. Unfortunately, a significant percentage of RT-treated patients develop locally persistent or recurrent tumours [3, 4].

Herbal medicine involves the treatment of disease based on the use of plants and plant extracts [5]. A large number of clinical studies have reported the therapeutic benefits of herbal medicines in combination with conventional therapeutics for the treatment of cancer patients [6-8]. Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is largely used in diets worldwide and also in traditional oriental medicine [9]. The compound [6]-Gingerol (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy-3-decanone) is the most abundant, biologically active constituent of

Ginger (Figure 1) [10]. [6]-Gingerol has been reported to have anti-oxidant, anti-inflammatory, and anti-tumour activities [9, 11-13]. In the present study, we investigated the anticancer properties of [6]-Gingerol in human PCa cells.

Materials and Methods

I Materials

The follow materials were used: Roswell Park Memorial Institute-1640 (RPMI-1640) media; foetal bovine serum (FBS); penicillin and streptomycin both purchased from Life Technologies (Waltham, MA USA); CellTiter 96[®] AQueous One Solution Cell Proliferation Assay (MTS) obtained from Promega (Madison, WI, USA); dimethyl sulfoxide (DMSO) and [6]-Gingerol purchased from Sigma Aldrich (Missouri, MO, USA); and LNCaP cells (CRL-1740[™]) obtained from American Type Culture Collection (ATCC[®]).

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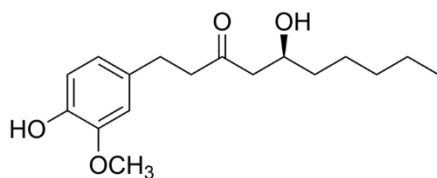


Figure 1: Chemical structure of the main active compound present in ginger extract ([6]-Gingerol) [10].

II Cell Culture

LNCAp cells were cultured in RPMI-1640 with 10% Foetal Bovine Serum (FBS) along with 100 U/ml penicillin and streptomycin at a concentration of 300 µg/mL. The cell line was maintained at 37°C in a humidified atmosphere of 5% CO₂ and were sub-cultured twice weekly.

III Cell viability (MTS)

Cell viability was assessed by using a [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium (MTS)]-based assay. The assay was based on the reduction of tetrazolium salt through the mitochondrial dehydrogenase of intact cells into a purple formazan product. The cells were seeded into 96-well plates (2.5×10³ cells/well), and incubated for 5-6 hr to facilitate attachment. Cells were then treated with 150 and 300µg/mL of [6]-Gingerol or vehicle alone (0.1% DMSO) in serum containing media, and incubated for 24hr at 37°C. After incubation, MTS solution was added to the plate at a final concentration of 0.5 mg/mL. The cells were incubated for 2 hr in the dark at 37°C. The resulting MTS-products were determined by measuring the absorbance at 490 nm with an ELISA reader.

IV Clonogenic assay

The clonogenic assay was performed to evaluate *in vitro* cell survival following treatment with [6]-Gingerol. For the colony formation assay, LNCAp cells (1×10² cells/dish) were divided into treatment groups with 300µg/mL of [6]-Gingerol and no treatment and seeded into 60 mm culture dishes, followed by incubation at 37°C. Ten days later, cell colonies were fixed and stained with methanol 20% and crystal violet 0.5% and colonies of at least 50 cells were counted.

V Radiosensitivity measurements

The LNCAp cells were seeded at a concentration of 100 – 2400 cells/dish and were divided into two groups: cells which served as irradiated controls and cells treated with [6]-gingerol and irradiated. Cells were irradiated by a ⁶⁰Co source in the range from 4 to 15 Gy, using the GammaCell 220 – Irradiation Unit of Canadian-Atomic Energy Commission Ltd. (CTR-IPEN). After 14 days of culture in normoxic conditions, cell colonies were fixed and stained with methanol 20% and crystal violet 0.5%; colonies of at least 50 cells were counted. The surviving fraction was calculated as the ratio of the plating efficiency of treated cells to the control cells. The dose enhancement ratio (DER) was calculated as the dose (Gy) that yielded a surviving fraction of 0.03 for control divided by that for the [6]-Gingerol treated cells.

VI Statistical Analysis

The results are presented as the mean ± S.E. Single comparisons of the mean values were completed via a Student's t-test. Multiple comparisons were assessed by One-way ANOVA, followed by Bonferroni's tests with GraphPad Prism version 6.0 software. A p-value < 0.05 was considered statistically significant.

VII Results and discussion

Radiotherapy is frequently combined with prostatectomy to treat localised tumours, but many patients present with recurrent or persistent disease [14]. One approach to improve the efficacy of RT is the use of radiosensitizers. The use of natural compounds as radiosensitizers could be a good therapeutic tool in oncology [15, 16].

In this work, we first investigated the effect of [6]-gingerol on viability of LNCAp prostate cancer cells. Our results demonstrated that [6]-Gingerol treatment induced a dose-dependent decrease in the cell viability (Figure 2). Compared with the vehicle control, the cell viabilities were 79.90 ± 3.56% and 53.06 ± 7.82% when the LNCAp cells were exposed to 150 µg/mL and 300 µg/mL of [6]-Gingerol, respectively. The inhibitory effect on cell viability was more prominent at a dose of 300 µM of [6]-Gingerol after 24-h of pre-treatment (P<0.001). A significant difference in cell viability was also observed between the cells treated with 150 µg/mL and 300 µg/mL of [6]-Gingerol (P<0.01), suggesting a possible [6]-Gingerol dose-dependent effect on LNCAp cancer cells.

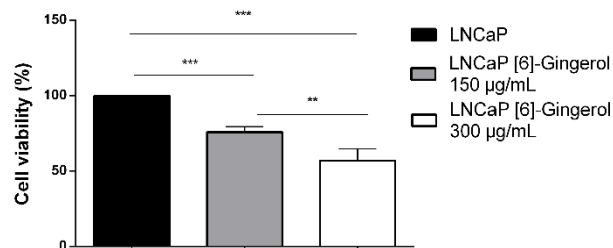


Figure 2: [6]-Gingerol treatment induced a decrease in the viability of LNCAp cells. Concentrations of 150 µg/mL and 300 µg/mL doses demonstrated significant reduction in cell counts ***P<0.001 vs control. Moreover, a significant difference in cell viability between the cells treated with 150 µg/mL and 300 µg/mL of [6]-Gingerol (**P<0.01). Each bar represents means ± SE, n=6. Statistical analysis was performed using One-way ANOVA followed by Bonferroni's test.

We further investigated the effects of 300 µM of [6]-Gingerol treatment on the drug sensitive and radioresistance assays. The drug sensitive effect of [6]-Gingerol on LNCAp cells was determined by using a colony formation (clonogenicity survival) assay. This assay has been previously employed for the evaluation of drug sensitivity in tumour cell lines [17]. After a 10-day culture period, colonies were stained and counted (Figure 3A). The efficiency of colony formation was 75.11± 5.07% when the LNCAp cells were exposed to 300 µg/mL of [6]-Gingerol. (P<0.05) (Figure 3B). These results indicate that [6]-Gingerol decreased the ability of LNCAp cells to form and sustain cell proliferation. A similar effect of [6]-Gingerol was also observed in pancreatic cells [18].

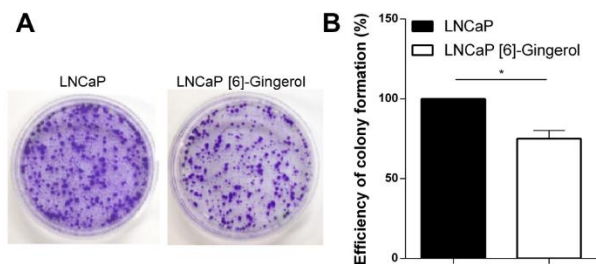


Figure 3: [6]-Gingerol decreased the clonogenic capacity of LNCaP cells. (A) Representative images of stained colonies (B) Quantitative analysis revealed a significant difference between control and the treated group (* $P < 0.05$). Each bar represents means \pm SE, $n = 3$. Statistical analysis was performed using Student's T test.

To analyse the effectiveness of [6]-Gingerol as a radiosensitizer, LNCaP cells were treated with 300 $\mu\text{g}/\text{mL}$ of [6]-Gingerol and irradiated at doses ranging from 4-15 Gy. Cells were grown for 14 days, after which the colonies were fixed and stained with crystal violet (Figure 4A). As shown in (Figure 4B), [6]-Gingerol induced a dose-dependent reduction on clonogenic survival. The results show that pretreatment with [6]-Gingerol significantly enhanced the cell killing effect of irradiation with a DER of 1.25, indicating its potential radiosensitizing effects in prostate cancer cells.

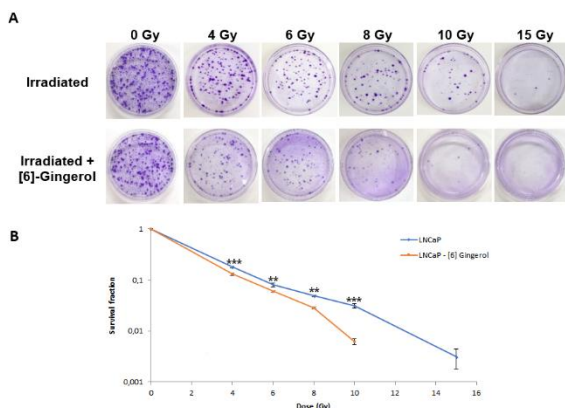


Figure 4: Radiosensitization of LNCaP cells. (A) Representative images of stained colonies. (B) Radiation cell survival curves of LNCaP cells with and without 300 $\mu\text{g}/\text{mL}$ [6]-Gingerol treatment. Clonogenic assays show that sensitizes [6]-Gingerol to irradiation-induced cell killing. (** $P < 0.01$, *** $P < 0.001$) Each bar represents means \pm SE, $n = 3$. Statistical analysis was performed using One-way ANOVA followed by Bonferroni's test.

Taken together, our results indicate [6]-Gingerol has anti-tumour activities on the LNCaP cell line. Further mechanistic studies are necessary and are currently in progress.

Acknowledgments

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Conflicts of interest

The authors declare that there are no conflict of interest.

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