Losartan sensitizes selectively prostate cancer cell to ionizing radiation

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Abstract: Losartan is an angiotensin II receptor (AT-II-R) blocker that is widely used by human for blood pressure regulation. Also, it has anti-tumor property. In this study, we investigated the radiosensitizing effect of losartan on cellular toxicity induced by ionizing radiation on prostate cancer and non-malignant fibroblast cells. Human prostate cancer (DU-145) and human non-malignant fibroblast cells (HFFF2) were treated with losartan at different concentrations (0.5, 1, 10, 50 and 100 µM) and then these cells were exposed to ionizing radiation. The cell proliferation was determined using MTT assay. Our results showed that losartan exhibited antitumor effect on prostate cancer cells; it was reduced cell survival to 66% at concentration 1 µM. Losartan showed an additive killing effect in combination with ionizing radiation on prostate cancer cell. The cell proliferation was reduced to 54% in the prostate cancer cells treated with losartan at concentration 1 µM in combination with ionizing radiation. Losartan did not exhibit any toxicity on HFFF2 cell. This result shows a promising effect of losartan on enhancement of therapeutic effect of ionizing radiation in patients during therapy.

Key words: Losartan, angiotensin II receptor, anti-proliferation, ionizing radiation, prostate cancer, radiosensitive effect.

Introduction

Ionizing radiation is widely used for treatment of cancers. In this effective treatment strategy, ionizing radiation produces free radicals and reactive oxygen species (ROS) in cellular and tissues environments. ROS are toxic and can attack to critical macromolecules such as DNA, RNA and peptides and alter these chemical structures and lead to cell dysfunction and death (1, 2). However, most tumor cells die when were exposed to ionizing radiation (IR); tumor cells can activate signal transduction pathways and increase the expression of survival proteins, which is result in tumor cell resistance to IR. For enhancement of cancer treatment, blockage of cell survival cascades could increase cancerous cell death to IR (3-5). It is important to notice that the blockage of these survival signals should be selectively on cancer cells and have minimized radiosensitizing effect on normal tissue.

Angiotensin II receptor (AT-II-R) is a major component in the renin–angiotensin–aldosterone system, it is involved in the blood pressure regulation. AT-II-R is overexpressed in tumor cells and plays a critical role in cell migration, proliferation and angiogenesis in cancers. Losartan as an AT-II-R blocker was used for inhibition of tumor growth (6, 7). Losartan improved drug and oxygen delivery to tumors and increased chemotherapy efficacy in breast and pancreatic cancer models (8). Several studies reported the radioprotective effects of AT-II-R inhibitors on normal organs such as kidney (9, 10).

However, losartan exhibited protective effects on normal tissues against IR-induce toxicity (11-13); its effect is unclear on cancer cell exposed to IR. To further explore the beneficial effects of losartan, the aim of this study was to investigate the therapeutic effect of losartan on the cell death induced by IR in prostate human cancer and also human non-malignant fibroblast cells in vitro.

Materials and Methods

Chemicals

Thiazolyl blue tetrazoliumbromide (MTT) was from Sigma (USA). Isopropanol and hydrochloric acid were purchased from Merck (Germany). Losartan was from Darupaksh Pharmaceutical Company (Iran). Roswell Park Memorial Institute (RPMI) medium, fetal bovine serum (FBS), penicillin, trypsin with EDTA, DMEM (Dulbecco’s modified eagle’s medium) medium and streptomycin were from Gibco (Paisley, UK). Plastic disposable tissue culture dishes and tubes were purchased from Jetbiofil (China). Losartan was dissolved in sterile water.

Culture of cells

Human prostate cancer (DU-145) and human non-malignant caucasian foetal foreskin fibroblast (HFFF2) cell lines were purchased from the Pasteur Institute of Iran. DU-145 and HFFF2 were cultured in RPMI and DMEM containing 10% fetal bovine serum and 100
μg/mL penicillin-streptomycin. Cells were incubated at 37°C in a humidified atmosphere (5% CO2).

**Irradiation**

In this study, the phantom was a custom-built 12×19×23 cm³ cubic phantom. This phantom was machined out of Plexiglass as 6 separate 2×19×23 cm³ blocks that one of them had an appropriate cutout (1.7×12.5×8.4 cm³) to accommodate the 96-well plate used in this work. Wells are arranged in eight columns and 12 rows. For irradiation of samples (cultured cells), the 96-well plate was embedded centrally within the phantom 4 cm from above and 6.3 cm from bottom of the phantom in its accommodated place. Cells were irradiated with 6 MV X-ray produced by a radiotherapy machine (Linear accelerator, Siemens, Primus, Germany) at a 1.96 Gy/min dose rate and source to sample distance (SSD) of 60 cm. Dose of irradiation was 6 Gy. For each dose, control cells were simultaneously exposed to same radiation. Radiation dose was determined by prior optimization.

**Antiproliferation assay**

DU-145 and HFFF2 cells (20,000 cells) were plated in each well of a 96-well plate and were incubated and allowed to attach for 24 h in a humidified atmosphere of 5% CO₂ in air at 37°C (Incubator-Biotek-NB 203L Korea). After incubation, cells were treated with various concentrations of losartan (0.5, 1, 10, 50 and 100 µM) and incubated for 2 h before exposure to IR. Losartan was dissolved in small volume of sterile water and then diluted with medium. The cells were incubated for 48 h in a humidified atmosphere of 5% CO₂ in air at 37°C. After 48 h, the culture medium was removed and MTT solution (5 mg/ml PBS) was then added and the plate was located in optimal atmosphere at 37°C. The metabolically active cells reduced MTT to blue formazan crystals. After incubating for 4 h, the formazan crystals in each well were dissolved in isoproponol (0.1% HCl). The absorbance of each well was read with an ELISA Reader at 570/630 nm (Bioteck, USA). Cells without any treatment were used as control for comparison of absorbance and cell viability.

**Statistical analysis**

All data are presented as mean ± SD from at least three separate experiments. One-way ANOVA with Tukey post test was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA. All P-values <0.05 were considered to represent significant differences.

**Results**

**Effect of losartan and ionizing radiation on cell proliferation in DU-145**

The effect of losartan on cell proliferation in DU145 was determined by MTT assay. Prostate cells proliferation was significantly inhibited by losartan at all tested concentrations after 48 h incubation (p<0.01) (Figure 1). Losartan exhibited a reduction of 27%, 34%, 31% and 28% in cellular growth in DU-145 cells when cells treated with 0.5, 1, 10, 50 and 100 µM of losartan, respectively. However, it was not observed a dose-cell viability manner in the cancer cell treated with losartan. Figure 1 shows the percentages of cell proliferation in the prostate cancer cells were treated by losartan. The additive effect of losartan with X-ray was observed on the percentage of cell proliferation in control, losartan-pretreated and/or X-ray in prostate cancer cells. Ionizing radiation reduced viability rate in the prostate cancer cell by 80% (p <0.05). The proliferation of prostate cancer cells were significantly reduced by losartan in combination with IR. Losartan significantly reduced percentage of cell viability to 54% and 60% at concentrations 1 and 10 µM, respectively (p<0.05). These results indicate that losartan has additive effect with X-ray on inhibition of cell growth in prostate cancer cell. It was observed a radiosensitizing effect by losartan on prostate cancer cells.

**Effect of losartan and ionizing radiation on cell proliferation in HFFF2 cells**

In the comparison with cancer cell, human non-malignant fibroblast cell (HFFF2) was used for cell proliferation effect of losartan It was not observed any statistically difference between concentrations of losartan for inhibition of cell growth at 48 h incubation in HFFF2 cells. Losartan did not exhibit any significantly cellular toxicity on HFFF2 cells (Figure 2). The additive effect of losartan with X-ray was evaluated on the percentage of cell proliferation in control, losartan-pretreated, and/or X-ray in HFFF2 cells. It is interesting that losartan did not exhibit any toxicity on HFFF2 cells in combination with X-ray.

**Discussion**

In this study, losartan exhibited a radiosensitizing effect on prostate cancer cell. Losartan reduced cell growth in combination with IR. Losartan did not reduce the cell growth in the non-malignant fibroblast...
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2007 Effect of losartan (L) at different concentrations (0.5, 1, 10 50 and 100 µM) alone and on non-malignant fibroblast cell (HFFF2). Cell proliferation was assayed with MTT test after 48 h incubation. No significant was observed between losartan-treated groups with control.

Our findings showed that losartan is a promising drug in patients on radiation therapy with radiosensitizing of prostate cancer cells in combination with IR.

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