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Original Research

Losartan sensitizes selectively prostate cancer cell to ionizing radiation

H. Yazdannejat[1,2], S. J. Hosseinimehr[1*], A. Ghasemi[3], T. A. Pourfallah[4], A. Rafiei[5]

[1] Department of Radiopharmacy, Faculty of Pharmacy, Pharmaceutical Sciences Research Center, Mazandaran University of Medical Sciences, Sari, Iran
[2] Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran
[3] Department of Radiology and Radiation Oncology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[4] Department of Biochemistry and Biophysics, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[5] Molecular and Cell Biology Research Center, Department of Immunology, Mazandaran University of Medical Sciences, Sari, Iran

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 Pasture Institute of Iran. DU-145 and HFFF2 were cultured in RPMI and DMEM containing 10% fetal bovine serum and 100

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*** Corresponding author:** Seyed Jalal Hosseinimehr, Department of Radiopharmacy, Faculty of Pharmacy, Pharmaceutical Sciences Research Center, Mazandaran University of Medical Sciences, Sari, Iran. Email: sjhosseinim@yahoo.com

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 cm^3 cubic phantom. This phantom was machined out of Plexiglass as 6 separate 2×19×23 cm^3 blocks that one of them had an appropriate cutout (1.7×12.5×8.4 cm^3) 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% CO_2 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% CO_2 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.

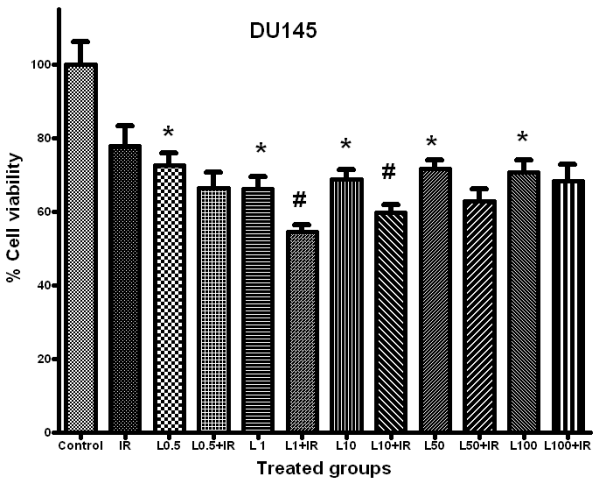

Figure 1. Effect of losartan (L) at different concentrations (0.5, 1, 10, 50 and 100 μM) alone and in combination with X-ray (IR) on prostate cancer cells DU145. Cell proliferation was assayed with MTT test after 48 h incubation.
$p<0.05$, comparison control group with IR group
* $p<0.01$, comparison with losartan (L) alone at all concentrations (0.5, 1, 10 50 and 100 μM) with control
#$p<0.05$, comparison L1+IR, 10+IR groups with IR group

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

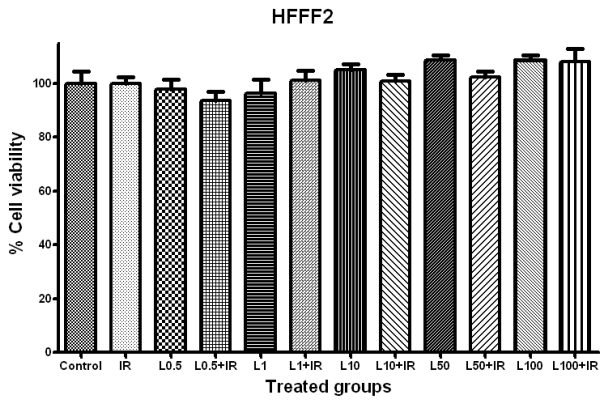

Figure 2. Effect of losartan (L) at different concentrations (0.5, 1, 10 50 and 100 μM) alone and on non-malignant fibroblast cell (HFFF2). Cell proliferation was assayed with MTT test after 48 h incubation.
No significant was observed between losartan-treated groups with control.

cell (HFFF2) exposed to X-ray. Losartan exhibited a selective radiosensitizing effect on the prostate cancer cells. The optimum antitumor and radiosensitizing effect of losartan was observed at concentration 1 μM. Plasma concentration of losartan is 233-611 ng/ml at a single dosage administration of 50 mg. Since molecular weight of losartan is 423, these concentrations are equal to 0.5-1.4 μM (14, 15). In this study, losartan did not exhibit a dose-cell anti-proliferation manner above concentration 1 μM. It is probably, this dose of losartan is sufficient to antagonize AT-II receptor, however, it is needed more experiments to explain exact mechanism. In this study, for assessment of cell proliferation, MTT assay was used. Cell proliferation is the increase in cell number as a result of cell growth. The MTT assay is based on the formation of dark-colored formazan dye by reduction of the tetrazolium salt MTT by metabolically active cells. Absorbance readings are related to the number of live cells. MTT assay can be a surrogate of the clonogenic assay in order to determine survival of irradiated cancer cells (16). MTT assay is needed a short time for assessment of cell viability as compare to clonogenic assay. AT-II exerts many biological actions by binding to AT-II-R1, in addition to its cardiovascular effects (17). AT-II-R1 is overexpressed in the several cancers such as prostate cancers (18-20). It plays an important role in the tumor migration, metastasis, invasion and angiogenesis. AT-II activates various intracellular signaling involved in tumor growth. AT-II plays various roles in proinflammatory responses through activation oxidative stress to help survival of cancer cell (4, 21). It is clear that blocking of AT-II-R1 by inhibitors may become an additive cell killing effect on treatment of cancer by chemotherapy and/or radiotherapy. Takahashi *et al* reported the administration of AT-II receptor blockers attenuated prostate carcinogenesis in transgenic rat for adenocarcinoma of prostate model. Also clinical data showed a suppressing effect of AT-II antagonists on the progression of prostate cancer associated with serum prostate-specific antigen (PSA) decrease (22). DU -145 cell is a hormone-independent prostate cancer cell, immunohistochemistry and Western blot analysis showed that angiotensin receptors AT1 and AT2 are present in DU-145 cell. Angiotensin III (Ang-III) and Angiotensin II (Ang-II) could modulate cell migration and proliferation of this cell line (23). Ang-III is converted from Ang-II. Similarly to Ang-II, Ang-III activated the cell growth of prostate cancer. Olmesartan is a selective blocker of the AT-II-R1 receptor and is used as anti-hypertensive agent. Olmesartan suppressed the cell growth induced by Ang-III treatment in DU-145 cells. Western blot of the phosphorylation of MAPK activated by Ang-III treatment, phosphorylation of MAPK was activated in DU-145 cells. Simultaneously, the phosphorylation was inhibited when cells were treated with olmesartan (24). In radiotherapy strategy, IR produces free radicals in cellular environment that are toxic for cells and resulting in cell deaths. These ROS are not selective for killing of cancer cells and may affect on normal cells and causes side effects. Radioprotective agents are able to protect normal cells against cell toxicity induced by ionizing radiation (25, 26). Also, several tumor cells are going resistance to IR through activation of cellular pathways and overexpression of cell surface receptors. For improvement of cancer treatment by IR, it is possible to use a drug that inhibits tumor cell growth through blockage of survival signal pathways on cancer cells. It could approach an additive effect in combination of IR and survival cell receptor antagonist. It is cautioned this strategy should not be enhanced cell toxicity induced by IR on normal cells. In this study, losartan as an AT-II-R blocker sensitized human prostate cancer cells to IR. IR-induced cells deaths were more in cancer cells as compared with non-malignant cells treated with losartan. AT-II-R blocker exerts an additive cell killing effect with IR on prostate cancer cells. Future experiments will be required to understand the exact molecular mechanisms by which losartan contribute to cell killing effect on cancerous cells without any toxicity on normal cells. Also more details related to radiosensitizing effect of losartan, future experiments such as flow cytometry and Western blot are needed.

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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