Iranian Journal of Basic Medical Sciences

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IJBMS

Evaluation of the effects of paederus beetle extract and gamma irradiation on HeLa cells

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ARTICLE INFO

Article type:
Short communication

Article history:
Received: Jun 13, 2013
Accepted: Nov 14, 2013

Keywords:
Gamma irradiation
Pederine
Radioprotection
Radiosensitizer

ABSTRACT

Objective(s): Cervical cancer is a malignancy that is the second most common cause of death from cancer in women throughout the world. Paederus beetle (*Paederus fuscipes*) extract (PBE), contains bioactive compounds such as pederine which has cytotoxic properties and blocks DNA and protein synthesis at very low concentrations. In this investigation we tried to determine the effects co-treatment with PBE and gamma irradiation on HeLa cells.

Materials and Methods: The viability of the cells was measured by two methods: MTT and Colony assay.

Results: We found that supplementing gamma irradiation therapy with PBE does not increase cell death and it might even interfere with its cytotoxicty at the concentrations below 0.1 ng/ml and the viability for irradiation vs irradiation + PBE was 37%: 60%.

Conclusion: This finding might be due to radioprotective effects of the very low doses of PBE against gamma radiation.

► *Please cite this paper as:*
Samani F, Shabestani Monfared A, Zabihi E, Khafri S, Karimi M, Akhavan Niaki H. Evaluation of the effects of co-treatment with paederus beetle extract and gamma irradiation on HeLa cells. Iran J Basic Med Sci 2014; 17:303-306.

Introduction

Cervical cancer is a malignancy that is the second most common cause of death from cancer in women throughout the world and different types of treatment are used for its management (1). Based on the International Federation of Gynecology and Obstetrics (FIGO) division, cervical cancer is a neoplasia including 4 stages: stages I and II which are locally advanced and stages III and IV which are spread (metastatic) stages (2). Cervical cancer is generally managed by surgery, radiotherapy or chemo-radiotherapy (3).

For more locally advanced type of disease, according to the division system of (FIGO), radiotherapy is the first approach to its treatment. In radiotherapy, the normal tissue in conjunction with the tumor is damaged, for this reason the total dose for cancer treatment is limited. Thus, any combination that enhances the radiation therapeutic efficiency without dose modification would be valuable in this regard (4).

The most common drugs that can be used as radiosensitizer for the treatment of cervical cancer is cisplatin which might also be a sensitizer for hypoxic cells (5). Carboplatin, nedaplatin, camptothecins, paclitaxel, gemcitabine are other radiosensitizers that were employed in clinical or nonclinical trials (6). During the recent years, natural products have been developed to be used as anticancer agents. They have been used in natural or synthetic forms. Antibiotics extracted from microbes, such as bleomycin, actinomycin, mitomycin C, or compounds derived from plants such as bisindole alkaloids, epipodophyllotoxins , taxanes are some examples from the long list of natural antitumor compounds (7).

The haemolymph of Paederus rove beetles (*Paederus fuscipes)* contains bioactive agents which can lead to a particular kind of skin injury known as dermatitis linearis (8) . The most important chemical which is found in this insect's haemolymph is pederine. Other chemicals like cantharidin, pederon and pseudopederin seem to play negligible roles in Paederus beetle extract (PBE) cytotoxic effects (8). Pederin is a symbiotic compound which is produced by a kind of bacteria which has close relationship with *Pseudomonas aeruginosa* (9).

This compound has a non-protein structure (10) and cytotoxic properties which could kill cancerous

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cells in plants, rats and mice (8). According to macromolecular synthesis studies using radioactive precursors , pederine interferes with protein and DNA synthesis and eventually blocks their production but does not affect RNA synthesis (11). This compound also induces cell fusion in human skin fibroblasts, *in vitro* (12) .

Pederine, as the main cytotoxic component of PBE, has tremendous side effects when used systemically in human and despite of the numerous studies indicating its antitumor activity, no investigation has been performed concerning the effects of the combination of PBE with ionizing radiation on cancerous cell lines. Therefore, this study was designed to evaluate the potential effects of the combination of PBE and ionizing radiation on HeLa cells.

Materials and Methods

Cells and reagents

HeLa cell line was obtained from Iran Pasteur Institute, Tehran, Iran). The RPMI 1640 medium, fetal bovine serum (FBS) and PenStrep® (penicillin10000 IU +streptomycin 10 mg/ml) were all purchased from PAA Laboratories GmbH (Austria). Giemsa stain (0.4%) and MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) were purchased from Sigma Aldrich Co. (UK). The MTT powder was dissolved in PBS at the concentration of 5 mg/ml. Isopropanol (2-propanol), methanol and fuming hydrochloric acid (37%) were acquired from Merck Chemicals (Germany).

Irradiation

Cells were exposed to ^{60}Co γ-irradiation source from Theratone780 (ACL, Canada) at the dose rate of 1.01 Gy/min. The distance between the source and the plates (SSD) was 80 cm. Generally, radiation doses were radiated in two forms: (0.5, 1, 1.5 and 2 Gy) with 9×13 field size for colony assay and (1, 2 and 4 Gy) with 9×5 field size for MTT assay (13).

Paederus beetles extract preparation

About 1×10^3 adult insects (*Paederus fusipes curtis*) were collected, grinded and mixed in ethanol (99%). The mixture was put in a shaking incubator (37°C) for 2 hr. Then, the homogenate was centrifuged and after decanting, the supernatant was transferred into a petri dish and left to dry, overnight. The dried extract was dissolved in ethanol (99%) and diluted by RPMI-1640 to prepare different concentrations.

Proliferation tests

Viability test using MTT assay

Cells were seeded in 96-well plates (7×10^3 cells/well) and the MTT assay was performed after 24, 48, 72 and 96 hr treatment with different concentrations of either PBE (1.7, 34, 3.4×10^2 and 3.4×10^3 ng/ml) in the complete medium (RPMI-1640 supplemented with 10% FBS and 1% PenStrep®), different doses of gamma irradiation (1, 2 and 4 Gy) and the combination of both. To investigate combined effects of PBE and radiation, cells were treated with the above mentioned concentrations of the extract for 22 hr. Then, cells were exposed to radiation at the dose of 2 Gy. Finally, to perform MTT assay, 15 µl of MTT solution (5 mg/ml) was added into each well and the plates were incubated at 37°C for 3.5 hr. The precipitated formazan was dissolved in 200 µl of acidic isopropanol and the optical density was measured using a microplate-reader (Ryto RT-2100, China) at 570 nm wavelength.

Viability test using colony- forming assay

HeLa cells were cultured as triplicate in 6-well plates at the densities of 900 or 1200 cells/well with 4.5 ml culture medium. The plating efficiency in this method was about 20%. After 24 hr of incubation, the cells were treated with different concentrations of PBE (34×10^{-3}, 34×10^{-2}, 34, 3.4×10^2 and 3.4×10^3 ng/ml), different doses of radiation (0.5, 1, 1.5 and 2 Gy) or the combination of both for 24 hr. To investigate the combined effects of PBE and radiation, cells were treated with the above mentioned concentrations of the extract for 22 hr, and then cells were exposed to radiation at the dose of 2 Gy. The culture media were changed and the cells were incubated for the next 10 days. On day 12 (after plating the cells), the medium was removed and the cells were stained with Giemsa (0.4%) and the colonies with ≥ 50 cells were counted as survival colonies. Data analysis was done by ANOVA test and chefe's multi-comparisons.

Results

MTT assay

The proliferation of cells (excluding the lowest concentration) was repressed in a dose-dependent manner, by increasing the time of exposure to PBE and more than 48 hr cytotoxicity reaches its maximum effect ($0.001 < P\text{-}value \leq 0.05$) .The viability of the cells was not significantly decreased after exposure to irradiation, except for 4 Gy at 96 hr ($0.001 < P\text{-}value \leq 0.05$). Since the dose of 2 Gy routinely use in radiotherapy, we selected 2 Gy of radiation to be used with different concentrations of the extract. The proliferation of the cells were repressed at a dose-dependent manner, MTT assay showed that the viability of the cells treated with the combination of radiation and PBE was significantly lower than those treated with IR alone after 48 hr, but not significantly lower than those treated with PBE alone.

Colony – forming assay

Survival fractions of HeLa cells were decreased by increasing doses of gamma radiation and gamma

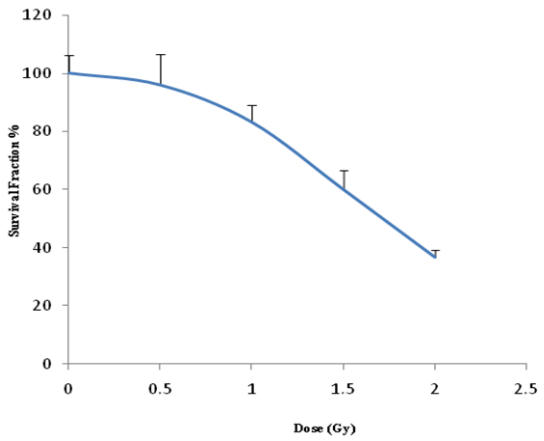

Figure 1. Survival curve of HeLa cells exposed to gamma radiation

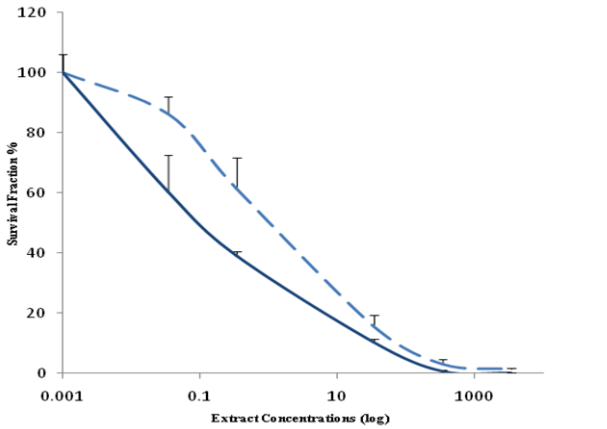

Figure 2. Survival curve of HeLa cells exposed to Paederus beetle extract (- - -) and combination of Paederus beetle extract and gamma radiation (_)

radiation at doses higher than 1 Gy caused significant reduction in survival fraction compared to control group as shown in Figure 1. Survival fractions of HeLa cells were decreased by increasing concentrations of PBE and except for the lowest concentration of PBE, the viability was significantly lower than the control group, (Figure 2). Survival curve of HeLa cells treated with the combination of different concentrations of PBE and 2 Gy radiation, did not show significant difference compared to PBE survival curve. In general, survival fractions in combination of PBE and radiation compared to PBE alone, were not significantly low. Also, compared to radiation alone treatment, following the treatment with the combination of the lowest concentrations of PBE and radiation, more cells survived (Figure 2).

Discussion

Radiotherapy is the most common approach to cure cervical cancer, but combination of the drugs that have radiosensitizing effects with the radiation, have been always very important to treat this malignancy. This strategy lowers the total doses of radiation, so it might decrease the side effects of radiotherapy and enhance the treatment efficiency (4).

In this study, the effect of the combination of PBE and gamma radiation on HeLa cells was investigated. According to our results, PBE does not show any statistically significant radiosensitizing property. In this study, MTT assay was applied to find out the acute effects and the colony assay was performed to investigate delayed effects of drug and ionizing radiation on HeLa cells.

The results obtained from MTT assay showed that PBE has cytotoxic effects at low concentrations (approximately 30 ng/ml). Similar results to our ones have been published on the cytotoxicity of pederin on HeLa cell lines, using different test procedures (14). Based on our results, gamma radiation does not lead to statistically significant reduction of cell proliferation at the doses which are usually used in the conventional radiotherapy (2 Gy) at least for 96 hr after exposure. Colony forming assay is a worthwhile method to perform radiobiological examinations such as evaluation of ionizing radiation effects on the cells with or without drugs (15). Results obtained from colony assay showed that the gamma radiation at doses higher than 1 Gy has antiproliferative effects on HeLa cell line. Also, based on the data obtained from this method, PBE is a strong inhibitor of cell proliferation which can cause total mortality of the cell population at the concentrations below 1 µg/ml.

Pederine is a natural compound which exists in coleopter *P. fuscipes* extract (11). It is a bioactive agent that causes fibroblasts fusion (12), has antibacterial properties and suppresses the growth of mammalian cell lines at low concentrations (8, 10). Since the PBE cytotoxicity mechanism is not clear yet, we showed that PBE at low concentrations can inhibit cell proliferation. This finding highlights the potential of pederine (the cytotoxic component of PBE) as a natural product to treat cancerous cell lines. Combination of PBE and gamma radiation against HeLa cells, as illustrated above, did not have additive or synergism impact as compared with each factor alone. Also, an increase in viability was observed when the combination of PBE (at the concentrations below 0.1 ng/ml) and gamma radiation was employed. It seems that at very low concentrations of PBE, the amount of pederine decreases and not only causes less cytotoxicity but also can protect cells from ionizing radiation effects.

It appears that PBE lessens the antiproliferative effects of radiation and it may save cells from damages caused by ionizing radiation on biological systems. However, this claim needs more investigations on the mechanism of action of other compounds existing in PBE to justify its radioprotecting properties.

Conclusion

According to our results, PBE in combination with the gamma radiation did not result in radiosensitization. Also, results showed that PBE at very low concentrations might be a radioprotectore chemical but this finding needs further investigations.

Acknowledgment

The authors would like to thank Mr Mohammad Abdollahpour for his valuable assistance. The authors are also grateful to the staff of radiotherapy department of Shahid Rajaii Hospital, Tehran, Iran. Especial thanks to Mr Rezaei and Mr Mokhtarpoor for their kind assistance.

Conflict of Interests

All authors declare that they have no conflicts of interest.

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