NATURAL PLANT PRODUCT BERBERINE/CISPLATIN BASED RADIOTHERAPY FOR CERVICAL CANCER: THE NEW AND EFFECTIVE METHOD TO TREAT CERVICAL CANCER

Komal¹, Singh Mayank², Deshwal Vishal kumar³*

¹, ² Department of Biochemistry, All India Institute of Medical Science, New Delhi, India.
¹, ³ School of Life Sciences, Singhania University, Pachri Bari, Rajasthan, India.
*Corresponding Author: E-mail: vishal_deshwal@rediffmail.com; Mobile: +919897538555

ABSTRACT

Cervical cancer is the site of excessive inflammation which leads to extensive DNA damage and thus promotes carcinogenesis. Existing treatment regime for cervical cancer is radiotherapy along with platinum based drugs like cisplatin and carboplatin but it is associated with various side effects to normal cells and problem of radio-resistance. Berberine is a natural chemo-preventive agent extracted from Berberis aristata that has been shown to suppress and retard carcinogenesis by inhibiting inflammation. In this study we compared the cisplatin based radiotherapy with a Berberine/cisplatin based radiotherapy in cervical cancer in vitro. Treatment of cervical cancer cell lines SiHa and CaSki with Berberine/cisplatin combination followed by treatment by ionizing radiation (IR) resulted in increased apoptosis in comparison to cisplatin based radiotherapy. The combination therapy of Cisplatin/Berberine/IR resulted in upregulation of pro apoptotic proteins like Bax, p73 and down regulation of anti apoptotic Bcl Xl, inflammatory Cox 2, Cyclin D1 accompanied by increase in activity of Caspase -9 and -3. Reduction in Telomerase activity was also seen in all the HPV positive cells.

KEYWORDS: Cervical cancer, Radiotherapy, Berberine, Cisplatin

Cite this article:
INTRODUCTION

Cervical cancer remains one of the major cancers amongst women worldwide with a high rate of mortality (Ciesielska et al., 2012). Radiotherapy in combination with cisplatin remains the treatment of choice in majority of cases in which the cancer is locally advanced. Currently the chemotherapeutic drugs cisplatin and 5-Fluorouracil are used as radio-sensitizers along with ionizing radiation (IR) for the treatment of cervical cancer (Rosa et al., 2012). Nevertheless, therapeutic results are far from optimal, so new and safer therapeutic combinations need to be investigated which specifically target cervical cancer cells with minimal toxicity to normal tissue. Although chemo radiotherapy is more effective as compared to radiotherapy alone, it is associated with dose limiting toxicities like gastrointestinal and hematological toxicities (Tan et al., 2012). Natural products offer an excellent alternative for therapeutic use as opposed to synthetic compounds because of their relatively well established safety profile (Deshwal, 2012; Kumar et al., 2012; Makhloufi et al., 2012). Several natural products are being tested as potential radio sensitizers. Berberine is a natural compound that allows prevention, suppression and retardation of carcinogenesis. Berberine [1, 7-bis-(4 -Hydroxy-3-methoxyphenyl) -1, 6 heptadiene -3, 5-dione] is a major constituent of product extracted from the rhizome of the plant Berberis aristata found in South and Southeast tropical Asia. Berberine (Plate 1) has been shown to be a potent chemo-preventive agent inhibiting tumor progression against skin, oral, intestinal, breast, colon and prostate cancer (Diogo et al., 2011). Berberine has been shown to confer radiosensitizing effect in prostate cancer cells, squamous cell carcinomas (Vuddanda et al., 2010; Tillhon et al., 2012) and recently in HeLa and SiHa, human cervical cancer cells (Javvadi et al., 2008). The major problem with cervical cancer is that the cancer cells become increasingly radioresistant due to activation of various antiapoptotic genes/cascades resulting in therapy failure, and the standard chemo-radiotherapy regime is unable to address this problem (Aggarwal et al., 2006). The goal of the present study was to compare the standard chemo-radiotherapy regime comprising of cisplatin/IR with the cisplatin/Berberine/IR combination therapy and assess its effect on protein which confers radio resistance to cervical cancer cells in vitro.

Plate 1: Chemical structure of Berberine
MATERIAL AND METHODS

Cell culture and chemicals

Human cervical cancer SiHa and Ca Ski cells were obtained from National Centre for Cell Sciences, Pune, India and were maintained in either Dulbecco’s modified Eagle’s medium or RPMI1640 (Sigma, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (HyClone), antibiotics, in a humidified atmosphere of 95% air and 5% CO$_2$ at 37ºC.

Cells were exposed to varying Ionizing radiation (IR). Antibodies against p73, Bcl xl, Bax, Cyclin D1, AIF and Cox 2 as well as secondary AP conjugated antibodies were obtained from Santa Cruz, USA. Berberine was obtained from Sigma, USA.

Flow cytometry

Cells (1 × 10$^4$ cells) were treated with 50 μM and 75 μM Berberine for 24 hrs and then harvested. Cells were fixed in 70% ethanol and left overnight at −20°C. Cells were then washed with PBS and incubated in staining solution (20 μg/ml propidium iodide, 50 μg/ml RNAse, 0.1% Triton X-100 and 0.1 mM EDTA) for 2 hrs at 4°C in dark. The DNA content of the cells was measured by flow cytometer (Becton Dickenson, USA) using Diva software.

Assay of telomerase activity

This was measured using the PCR-ELISA kit based on TRAP (Telomerase repeat amplification protocol) assay (Roche Molecular Bio-chemicals, Germany). The samples were lysed and an aliquot containing 2μg protein was used for the assay. Telomerase positive embryonic kidney cell line (HEK-293) was used as positive control while heat inactivated HeLa extract was used as negative control. The telomerase activity was detected and expressed as relative units (RU) (Khanna et al., 2003).

Western blot analysis

The level of expression of various proteins was determined in control and treated cells by Western blotting as described previously (Singh et al., 2007). Briefly, cells were washed twice in PBS (Phosphate buffered saline) and lysed in RIPA lysis buffer. Total protein was determined by the Bradford assay. Equal amount of protein was loaded and run on 10–15% SDS-polyacrylamide gel. The proteins were transferred to a nitrocellulose membrane. The membrane was blocked with 5% BSA (Bovine serum albumin), followed by hybridization with respective primary and secondary antibody. Final detection was performed with BCIP/NBT BCIP (5-bromo-4-chloro-3'-indolyphosphate p-toluidine salt/ NBT (nitro-blue tetrazolium chloride), substrate (Promega, USA). The bands were analyzed and quantified using α image scanner densitometer and normalized with β actin control. The density of control was taken as 1 and results of treatment were expressed in relation to the control as relative unit (RU).

Assay of Caspase -3, -9 activities

Caspases-3 and -9 activity were measured by the direct assay for Caspase enzyme activity in the cell lysate using synthetic fluorogenic substrate (Ac-DEVD-AFC; substrate for caspase 3; MBL Bioscience, USA; Ac-LEHD-AFC, substrate for Caspase 9; Genotech, USA) as described by the manufacturer. Amount of fluorogenic AMC/AFC moiety released was measured using a spectro-fluorimeter (ex.380 nm, em.420–460 nm for Caspase -3; ex.400 nm, em.490–520 nm for Caspase-9). The results are expressed in arbitrary fluorescence units/mg protein (Singh and Singh, 2008).

Statistical analysis

Results were expressed as mean of three individual experiments ± standard deviation which was calculated using Microsoft excel.
RESULTS

Effect of ionizing radiation (IR) on cervical cancer cells

For treatment with ionizing radiation HPV positive cell lines were chosen i.e HPV 16 positive cell lines SiHa, CaSki which vary in their HPV copy number. Cells were treated with ionizing radiation doses ranging from 1Gy to 3Gy, to assess their effect on these cell lines. Gradual increase in apoptosis was found on increasing radiation dose from 1Gy to 3Gy (Fig 1) but the sensitivity to ionizing radiation varied from one cell line to another demonstrating the variation of resistance to radiation with cell type. Our data indicates that Ionizing radiation dose of 1Gy results in 15.5% apoptosis in SiHa and 18.5% apoptosis in CaSki cells. Treatment with 2 Gy radiation dose resulted in increase in apoptosis in both the cell lines, in which SiHa showed 23.6% and CaSki showed 19.08% apoptosis respectively, hence demonstrating the radio-resistance. Escalation of radiation dose to 3Gy resulted in marginal increase in apoptosis 26.23% in SiHa and 22.04% in CaSki cells. Since there was only a marginal increase in apoptosis on increasing radiation dose from 2Gy to the dose of 3Gy, hence we settled for the radiation dose of 2Gy for all the experiments. This dose was used in combination treatments with cisplatin and Berberine also.

Effect of Cisplatin and Cisplatin / IR on cervical cancer cells

Platinum compounds like cisplatin in combination with radiotherapy are used extensively for treatment of cervical cancer so we tried to mimic this situation in vitro on the cell lines. First we assessed the effect of cisplatin alone on these cell lines and in combination with IR (2Gy). Treatment of SiHa and CaSki with 5μg/ml cisplatin for 24 hrs resulted in 19.4% and 12.95% apoptosis respectively (Fig 2). Coupling this dose of cisplatin with 2 Gy ionizing radiation (IR) resulted in increase in apoptosis to 23.4% and 16.74% in SiHa and CaSki cells respectively. Escalation of cisplatin dose to 10 μg/ml for 24 hrs resulted in increase in apoptosis in SiHa and CaSki to 32.65% and 35.4%, whereas in combination with 2Gy IR it increased to 35.25% and 38.42%. Thus a chemoradiation dose of 10 μg/ml CP (cisplatin) / 2Gy IR was selected for further experiments (Fig 2).

Effects of Berberine / CP / IR on cervical cancer cells

Berberine has been established as a potential chemotherapeutic agent in various clinical trials and has been found to be well tolerated at higher doses but its bio-availability remains a major problem. Howell et al., (2007) have summarized based on in vitro and in vivo studies and clinical trials on natural products like Curcumin and Berberine. It is shown that the concentrations of natural products that were achievable in the plasma of patients were only at a lower micromolar range; hence, they have suggested that for in vitro studies concentration of berberine in less than 50 μM range do not have any physiological relevance. The significant radio-sensitization achieved by the moderate dose of berberine at relevant doses in vitro (2–6 Gy) has promising implications for improving radiation therapy especially in radio resistant tumors such as the tumors of the uterine cervix. Hence we coupled the above dose of 10 μg/ml CP with 50 μM berberine. The cells were treated to this combination dose for 24 hrs followed by 2Gy IR. An abrupt increase in apoptosis was seen in the cell lines with SiHa showing 49.8% apoptosis which was higher than 35.25% apoptosis observed with 10 μg/ml CP/ 2Gy IR (Ionizing Radiation) dose, used as standard therapy. Similarly, CaSki showed an increase in apoptosis to 41.65% on treatment with the combination dose against apoptosis achieved on treatment with CP/ IR combination (Fig 2). Clearly our results show that Berberine is selectively increasing apoptosis in these cell lines. We then compared the effect of Berberine/ CP combination with CP/ IR, on various proteins involved in apoptosis both pro-apoptotic and anti-apoptotic, radio resistance and inflammatory response.
Microscopic and Flow cytometric analysis of apoptosis in SiHa (Fig 1A) and Ca Ski (Fig 1B) cells on treatment with IR (1-3Gy).

Microscopic and Flow cytometric analysis of apoptosis in SiHa (Fig 2A) and Ca Ski (Fig 2B) cells on treatment with CP/IR and 75µM Berberine/10μgm Cisplatin/IR for 24 hrs. The percentage Apoptosis shown in the bar diagram is mean ± SD of three individual experiments.
Effects of berberine / Cisplatin / IR and Cisplatin / IR on protein p73

The protein p53 is a well-known tumor suppressor protein that functions primarily as a transcription factor, initiates cell cycle arrest and apoptosis after genotoxic stress. Both the cell lines used in the current study are HPV (Human Papilloma Virus) positive cell lines which encodes for viral protein E6 which causes ubiquitin mediated degradation of p53 so another member of p53 family p73 becomes important in these cells. It has been shown that p73 plays an important role in Hydrogen peroxide induced apoptosis (Singh et al., 2007; Singh and Singh 2008). Since radiation induced apoptosis involves ROS (Reactive oxygen species) so we determined the effect of cisplatin/IR and cisplatin/Berberine/IR combination on p73 in these cell lines. In SiHa and CaSki cells there was 36% and 44% increase in p73 expression on treatment with cisplatin/IR but on treatment with Berberine/cisplatin/IR the expression of p73 increased to 54%, 98% (Fig.3B) demonstrating that p73 level changes in response to Berberine. Javvadi et al., 2008 have shown that ROS plays an important role in Berberine/IR mediated apoptosis in SiHa cells. Our results are in agreement with their findings and suggest that cisplatin/ Berberine /IR induced activation of p73 which may involve reactive oxygen species (ROS).

Effects of Berberine / Cisplatin / IR combination on cyclin D1

Cyclin D1 is involved in cell-cycle arrest in DNA-damage response. Cyclin D1 has been shown to be induced by low-dose ionizing radiation in human keratinocytes with an adaptive radio-resistance (Ahmed et al., 2008). On exposure of SiHa and CaSki to Cisplatin/IR there was a 22% and 30% decrease in level of cyclin D1 which was reduced to 49% in case of CaSki but remained unchanged in SiHa on treatment by Berberine/cisplatin/IR, thereby demonstrating variation from cell type to cell type (Fig.3A)

![Figure 3](image)

Effect on the level of Cyclin D1 (Fig 3A) protein and apoptotic p73 (Fig 3B) protein in SiHa and CaSki cells by western blotting. Lane 1 control, Lane 2 CP 10μg for 24 hrs followed by 2 Gy IR Lane 3 CP 10µg/75µM Berberine for 24 hrs followed by 2Gy IR (Relative unit, RU). The results shown are mean ± SD of three individual experiments in the bar diagram.
Berberine / Cisplatin / IR combination treatment resulted in activation of proapoptotic Bax and reduction in level of Bcl XL

Cells on exposure to IR along with chemotherapeutic agent results in DNA damage and if this is severe, p53 and its counterparts like p73 may trigger programmed cell death by means of pro-apoptotic genes such as Bax and inhibition of anti-apoptotic Bcl XL. It has been demonstrated that radio resistant laryngeal cancer was associated with increased Bcl-2 and Bcl-XL expression and loss of Bax expression. Bcl-2 family has been proposed to predict radiotherapy outcome (Nix et al., 2005). The association between expression of Bcl-2, Bcl-XL and Bax with radio resistant cancer suggests a potential mechanism by which cancer cells avoid the destructive effects of radiotherapy. We probed the effect of Berberine/Cisplatin/IR combination on level of Bax in SiHa and CaSki cells. The Bax expression increased to 45% and 52% on treatment with cisplatin/IR, but this increased to 46% and 68% with respect to control on treatment with Berberine/cisplatin/IR combination.(Fig.4A) There was a marginal increase in case of CaSki and nearly no change in case of SiHa. However, in case of anti-apoptotic Bcl XL we obtained a 7% decrease in its level in case of SiHa and 15% decrease in its level in case of CaSki cells on treatment with cisplatin/IR (Fig.4B). In contrast, on treatment with Berberine/cisplatin/IR we obtained a substantial decrease in level of BclXL i.e. 22% in SiHa and 26% in CaSki.

Berberine / Cisplatin / IR combination treatment did not affect the expression of COX 2 and AIF

COX-2 has been implicated in carcinogenesis of systemic cancers. COX-2 inhibition has been shown to increase the radio-sensitivity of various tumors. Results from the present study demonstrate that on treatment of SiHa and CaSki cells with cisplatin/IR there was a 36% and 13% increase respectively in expression of COX 2 but treatment of these cells with Berberine/cisplatin/IR resulted in 8% and 6% decrease respectively in level of COX2(Fig.5A). These results indicate that though Berberine/cisplatin/IR based therapy is repressing COX 2 the effect is only marginal and it varies with cell type. AIF expression was unaltered remaining nearly the same on both the treatments in both the cell lines (Fig 5B), suggesting its noninvolvement.

Berberine / Cisplatin / IR treatment enhances activation of both Caspase-3 and -9

Caspase -3 and -9 have been implicated to play an important role in mitochondrial mediated apoptosis by causing activation of Caspase activated DNAse, finally causing degradation of DNA. Hence we compared the effect of Berberine/cisplatin/IR and cisplatin/IR on Caspase -3 and -9 activities. The results show that in SiHa and CaSki cells there was a 1.19 and 1.23 fold increase in activity (Arbitrary fluorescence units, Afu converted to fold change compared to control) in activity of Caspase -3 on treatment with cisplatin/IR, while there was a 1.55 and 2.27 fold increase on treatment with Berberine/cisplatin/IR. Clearly Berberine is causing activation of caspase -3. To assess whether enhanced apoptosis was being mediated through mitochondrial pathway, we also studied the effect on caspase-9 activity. SiHa and CaSki cells showed 0.29 and 0.11 fold increase in activity of Caspase -9 on treatment with cisplatin/IR while there was a 0.38 and 0.78 fold increase on treatment with Berberine/cisplatin/IR. Thus Berberine appears to be mediating apoptosis through mitochondrial pathway (Fig 6B).
Fig 4

Effect on the level of antiapoptotic Bcl XL (Fig 4A) protein and apoptotic Bax (Fig 4B) protein in SiHa and CaSki cells by western blotting. **Lane 1** control, **Lane 2** CP 10μg for 24 hrs followed by 2 Gy IR, **Lane 3** CP 10μg/75µM Berberine for 24 hrs followed by 2Gy IR (Relative unit, RU). The results shown are mean ± SD of three individual experiments in the bar diagram.

Fig 5

Effect on the level of AIF (Apoptosis inducing factor) (Fig 5A) and inflammatory COX 2 (Fig 5B) protein in SiHa and CaSki cells by western blotting. **Lane 1** control, **Lane 2** CP 10μg for 24 hrs followed by 2 Gy IR, **Lane 3** CP 10μg/75µM Berberine for 24 hrs followed by 2Gy IR (Relative unit, RU). The results shown are mean ± SD of three individual experiments in the bar diagram.
Caspase-3 (Fig 7A) and -9 (Fig 7B) activity (Arbitrary fluorescence unit, Afu) after treatment with CP 10μg for 24 hrs followed by 2 Gy IR and 10μg/75μM Berberine for 24 hrs followed by 2Gy IR in SiHa and Ca Ski. The results are mean ± SD of three individual experiments.

**Fig 7**

Effect of 10μg CP for 24 hrs followed by 2 Gy IR and CP 10μg/75μM Berberine for 24 hrs followed by 2Gy IR on telomerase activity in SiHa and Ca Ski cells (RU). The results shown are mean ± SD of three individual experiments.

**Berberine / Cisplatin / IR combination causes reduction in activity of Telomerase with respect to Cisplatin/IR**

Telomerase activation plays a critical role in tumor growth and progression, in part by maintenance of telomere structure. Indeed, the ubiquitous expression of telomerase in human cancers makes telomerase a promising target for cancer therapy. We assessed the effect of Berberine/cisplatin/IR combination dose on telomerase with respect to cisplatin/IR combination dose by using PCR/ELISA methodology which assesses activity of hTERT. We observed that on treatment of SiHa and CaSki there was 22% and 39% decrease in telomerase with cisplatin/IR while there was an
82% and 75% decrease in telomerase activity on treatment with Berberine/cisplatin/IR (Figure 7). This data suggests that berberine based therapy causes substantial decrease in telomerase activity and this therapy can be a potent telomerase targeted approach for treatment of cervical cancer.

DISCUSSION

Cervical cancer remains one of the major killers amongst women worldwide. Chemo/radiotherapy regime which is cisplatin based radiotherapy is used for the treatment of advanced cervical cancer in India. Evidence shows that most of the chemotherapeutic drugs used in current clinical practice are radio-sensitizers. Several newer cytotoxic agents with radio-sensitizing properties are being tried in combination with cisplatin but their use is generally limited by dose related toxicities (Rosa et al., 2012). Natural products open a new avenue for treatment of cancer as they are generally tolerated at high doses. Animal studies have confirmed the anti-tumorigenic activity of natural products like Berberine and curcumin (Howells et al., 2007). Phase I clinical trials on curcumin showed that it is safe to humans up to 12,000 mg/day when taken orally (Cheng et al., 2001; Sharma et al., 2001; Lao et al., 2006) and caused histological improvement of precancerous lesions in patients, suggesting that it is biologically active at these doses (Cheng et al., 2001). Previous reports have indicated that Berberine confers radio-sensitizing effects in prostate and squamous cell carcinoma cell lines, and recently in cervical cancer cell lines HeLa and SiHa. Thus we evaluated the effect of Berberine in combination with cisplatin/IR and compared it with cisplatin/IR treatment. Our results suggest that Berberine/cisplatin/IR based therapy is likely to be more effective and safer to treat cervical cancer, as Berberine is well tolerated in humans, even at high doses. We present in vitro evidence that this approach targets radio-resistance and anti-apoptotic proteins in a much more specific way compared to the standard cisplatin/IR based therapy.

When cells are exposed to clinically relevant doses of ionizing radiation it causes DNA damage by generation of reactive oxygen species (ROS). This DNA damage causes a rapid ROS dependent activation of pro-apoptotic and anti-apoptotic cascade which involves activation of p53 family of genes acting downstream to trigger apoptosis if the damage is not repairable. The tumor cells are dynamic with respect to their reliance on specific cell signaling pathway to exist and rapidly adapt to repeated toxic challenges in an attempt to maintain tumor survival. Prolonged inhibition of any one of these pathways however, gives rise to lineage of cells which become resistant to inhibitor drug, with point mutations in the specific targeted proteins, or by reprogramming of multiple signaling processes within the cell (Valerie et al., 2007). So the need for today is therapies which target these multiple pathways and result in selective apoptosis of cancer cells and thus deal with the problem of radio-resistance. The cell lines used in this study are HPV positive, in which wild type p53 is targeted for ubiquitin mediated degradation so other members of p53 family like p73 become particularly important in these cell lines (Singh et al., 2007; 11 Singh and Singh 2008). We observed the activation of p73 in cisplatin/IR treated sample but this activation was much more in berberine/cisplatin/IR treated samples which indicates that combination of Berberine is causing much more activation of p73 probably through mediation of ROS. This has been demonstrated recently in some studies in which natural products like Berberine were shown to be a pro-oxidant in combination with ionizing radiation, and its radio-sensitizing properties were attributed to ROS mediated signaling (Javvadi et al., 2008).

Bax and Bcl XL are members of Bcl 2 family of proteins which control apoptosis and are associated with regulating the mitochondrial membrane permeability. Pro-apoptotic proteins like Bax by translocation from the cytosol to the mitochondria, induce cytochrome c release, whereas Bcl-XL exerts its anti-apoptotic activity, at least in part by
inhibiting the translocation of Bax to the mitochondria (Mohammad et al., 2008; Ow et al., 2008). Our results show a slight increase in Bax and a decrease in Bcl XL on Berberine treatment in both the cell lines. AIF (Apoptosis inducing factor) remained unchanged thereby suggesting its non involvement.

Caspases, a family of aspartic acid-specific proteases, are the major effectors of apoptosis. Once activated, caspases preside over the ordered dismantling of the cell through restricted proteolysis of hundreds of substrate proteins (Ow et al., 2008). Caspase -3 has been implicated in both the extrinsic and intrinsic pathway of apoptosis. Our findings show activation of caspase -3 on both the treatments but this activation was more marked on Berberine/cisplatin/IR treatment implicating that cells are undergoing apoptosis through caspase-3 mediated pathway. Similarly, caspase -9 was activated on both the treatments in both the cell lines, but was more marked on Berberine/cisplatin/IR treatment, implicating involvement of mitochondrial mediated apoptosis pathway. Next we assayed the effect of Berberine/cisplatin/IR on proteins involved in radioresistance like Telomerase, Cyclin D1 and Cox 2.

Cyclin D1 is involved in cell-cycle arrest in DNA-damage response. Cyclin D1 contributes to regulate G1 progression by forming a complex with different cyclin-dependent kinases. It has oncogenic properties and is frequently overexpressed in several human tumor types. Cyclin D1 has been shown to be induced by low-dose ionizing radiation in human keratinocytes with an adaptive radioresistance (Ahmed et al., 2008). Our results show a similar reduction in level of cyclin D1 on berberine treatment in both cell lines, but to different extents, demonstrating cell to cell type variation.

Cyclooxygenase-2 (COX-2), an enzyme induced by pro-inflammatory cytokines, mitogenic substances, oncogenes, growth factors, and hypoxia, among others, is involved in the metabolic conversion of arachidonic acid to prostaglandins in inflamed tissues and neoplasia. COX-2 is often overexpressed in malignant tumors and premalignant lesions. Because COX-2 may also be a determinant of tumor radio-resistance, its inhibition or inhibition of its products (prostaglandins) may improve tumor response to radiotherapy. Analyses of the effect of cisplatin/IR show that COX 2 was elevated in both the cells on treatment with cisplatin/IR indicating that this radio-resistant marker is elevated in all the cell lines however on treatment with berberine cisplatin/IR COX 2 was down-regulated in both the cell lines.

Telomeres and telomerase play a role in the regulation of the life span of the cell. Human cells express low levels of telomerase; however when telomere length reaches a critical level, abnormal activation of telomerase can lead to immortalization and uncontrolled proliferation (Agarwal et al., 2008; Gandellini et al., 2007). Our result shows that Berberine treatment caused a substantial reduction in telomerase activity in SiHa and CaSki cervical carcinoma cell lines. Thus Berberine is conferring a selective advantage over cisplatin based radiotherapy by causing marked decrease in telomerase activity.

In summary, our data provides in vitro evidence that supports the clinical importance of coupling Berberine with cisplatin as an efficient radiosensitizer for treatment of cervical cancer as it causes enhanced activation of p73 causing further down-regulation of anti-apoptotic Bcl XL and activation of caspase -3 and -9, resulting in enhanced apoptosis. We also provide evidence that Berberine/cisplatin based radiotherapy causes substantial down-regulation of telomerase activity, Cyclin D1 and COX 2, thus acting as a potent radiosensitizer. This data has clinical relevance as it highlights the importance of overcoming the problem of radio-resistance by specifically targeting radio-resistant proteins using Berberine which is well tolerated in human beings even at high doses, and thus promises to be effective drug in future clinical trials along with cisplatin/IR.
ACKNOWLEDGEMENT

We would like to acknowledge DBT for financial support to Komal. This work was also supported by SRF grant to Mayank Singh from CSIR. We also acknowledge the technical help from Jyotibasu for carrying out IR Treatment at IRCH, AIIMS.

REFERENCES


