RESEARCH ARTICLE

Inhibition of Proliferation of Cervical and Leukemic Cancer Cells by Penicillin G

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Abstract

Cancer, despite all the efforts, still causes one in five deaths worldwide. Surgery, chemotherapy and radiotherapy provide inadequate protection and instead affect normal cells along with cancer cells. The search for cancer cures from natural products (plants and animals) has been practice for over a decade and the use of purified chemical to treat cancer still continues. Several studies have been undertaken during last three decades to find the anti-cancerous property of various plant extract and toxins secreted by animals and micro-organism. These lead to the discovery of several promising molecule having anticancer activity, some of which are in clinical trial and may emerged to be a potential future drug in cancer therapy. In this study we have used penicillin to evaluate its anti-cancer activity. It shown significant effects at cellular and molecular levels against growth of HeLa and K562 cell lines.

Keywords: Chemotherapy - radiotherapy - toxins - penicillin - cell lines -

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Introduction

Cancer is an abnormal growth of cells resulted from series of multiple changes in expression of various genes which resulted in dysregulated balance of cell proliferation and cell death (Gibbs, 2003). These altered changes lead to a population of cells that metastasize to distant sites causing formation of new tumor (Klein, 2008). Cancer is one of the major global health problem. Currently, 1 out of 4 deaths in the world is due to cancer (Siegel et al., 2011). In India approx 555,000 people died of cancer in 2011. According to the recent finding, the two most common forms of cancers among the male population are oral (22.9%) and lung (11.4%) cancer. Whereas cervical (17.1%), stomach (14.1%) and breast (10.2%) cancers among the female (Dikshit et al., 2012). In the past decade there is a considerable progress in finding the ultimate cure by understanding the hallmark of cancer (Hanahan and Weinberg, 2011) and thereby for early detection and in various other treatment (Baskar et al., 2012). Although there are many anti- cancerous drugs available in the market but they do have their own side effects. The focus to find the cure has now shifted to natural resources. The use of natural product for the development of anticancer drugs is practiced throughout the world. Toxins from plants, animals and microbes act as potential bio resource and a therapeutic tool (Gomes et al., 2010). The antibiotic potential of Penicillin was first of all discovered by Alexander Fleming in 1929 (Diggins 1999). Penicillin is beta-lactam based antibiotic which is being developed as 'The Wonder Drug' for anti-bacterial therapy. With the recent advancements, antibiotics and their derivatives are found to have anticancer property and are been used as enzyme inhibitors (Xing et al., 2008). These beta- lactam based antibiotics inhibit the growth of tumor by DNA intercalation (Kuhn et al., 2004). According to a recent study N-thiolated monobactams induced apoptosis in many tumor cells but not in normal and non-transformed cell lines (Kazi et al., 2004). Ceftriaxone, with penicillin binding proteins as primary molecular targets, showed anti-tumor activity both in vitro and in vivo. It suppressed the growth of anchorage-independent cells by targeting Aurora B in A549, H520 and H1650 lung cancer cells. Similarly it also suppressed A549 and H520 lung tumor growth in xenograft animal model (Li et al., 2012). With this work we tried to evaluate the anti- cancerous property of Benzyl Penicillin, which is commonly known as Penicillin G, against cervical cancer cell line (HeLa) and leukemic cancer cell line (K562) respectively. The results were then compared with that of non-cancerous cell line (Vero).

Materials and Methods

Cell culture

HeLa, K562 and Vero cell lines were obtained from NCCS, Pune. Cells were cultured in DMEM in case of HeLa and Vero and RPMI 1640 for K562 and supplemented with 10% fetal bovine serum and penicillin/ streptomycin (100 unit/mL). Cell cultures were then maintained at 37°C in a humidified atmosphere with 5%CO_2. All fine chemicals used were obtained from

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Treatment of penicillin on cancer cells

Benzyl Penicillin or Penicillin G (12 lakhs units) was procured form Wyeth Pvt Ltd. Cell counting was done using the hemocytometer. Approximately 104 cells/well in case of Vero and HeLa and $2X10^4$ cells/well for K562 were loaded in 96-well plate. In case of HeLa and Vero, pre-treatment incubation of 24 hours was given which was not required for K562 as it is a suspension cell line. Different concentration of penicillin (10%, 7.5%, 5%, 2.5% and 1%), which was prepared in respective media, were added to the wells accordingly. The 96 well plate was then again kept in carbon dioxide incubator at 37°C and 5%CO_2 and effect of penicillin was observed at different time interval (3, 6, 12, 24, 48 and 72 hours).

MTT cell proliferation assay

For different time intervals (3, 6, 12, 24 and 48) 10 µl MTT solution was added to the wells and incubated for 2-4 hours 37^0C and 5%CO_2 until purple precipitate were visible. These precipitate were pelleted down through centrifugation and 100 µl of DMSO was added in order to dissolve the crystals. Absorbance was taken using an ELISA reader at 570 nm. The IC_{50} value was calculated using four parameter logistic curve.

Statistical analysis

One-way analysis of variance (ANOVA) was used for multiple comparisons followed by Newman-Keuls test. Differences with P values of <0.05 were considered statistically significant.

Gene expression

RNA was isolated from the control and treated cells. For HeLa, the concentration at which RNA was isolated was 2.5% at 24 hours and in case of K562, the concentration was 5% at 48 hours. RNA was quantified and cDNA was prepared using Qubit Flurometer. Gene expression was observed using Reverse Transcriptase-PCR. Genes targeted for this purpose were GAPDH, p53, MMP11 (for HeLa) and STAT5a (for K562).

Results

Effect of penicillin on cervical and leukemic cell line

The effect of penicillin on HeLa and K562 is shown in Figure 1 and 2 respectively. The triplicate study was conducted at different time interval of 6, 12, 24 and 48 hours respectively. Dose dependent study showed that 3 hours after the treatment was given, there was a significant change in the morphology of cell in 10% and 7.5% concentration for HeLa and K562. After 6 hours cells were in necrosis in both 10% and 7.5% concentration respectively in HeLa and K562. Cells in 5% concentration were seen under stress. After 24 hours, all the cells in 10%, 7.5% and 5% concentration were dead in HeLa. In 2.5% concentration, approx 50% cell were under necrosis. Whereas in K562, complete cell death was seen at 10% and 7.5%. In case of Vero, cell where healthy and dividing in 7.5% and 5% after 6hours and 12 hours of treatment whereas in case of HeLa and K562 cells were dead in mentioned concentration. This proves that a minimum of double strength concentration is required to kill non-cancerous cells.

MTT cell proliferation assay

The cytotoxic effect after the treatment of penicillin at different time interval was measured by reading the absorbance at 570 nm for HeLa and K562 cells. 2.5% concentration of penicillin at time point of 24 hours and 5% penicillin at time point of 48 hours showed significant effect in case of HeLa and K562 cells respectively. Therefore, these concentrations were only used for further evaluation at molecular level. With the help of One-way analysis of variance (ANOVA), statistical analysis was done and p values were obtained as shown in Figure 3 (HeLa cells) and Figure 4 (K562 cells).

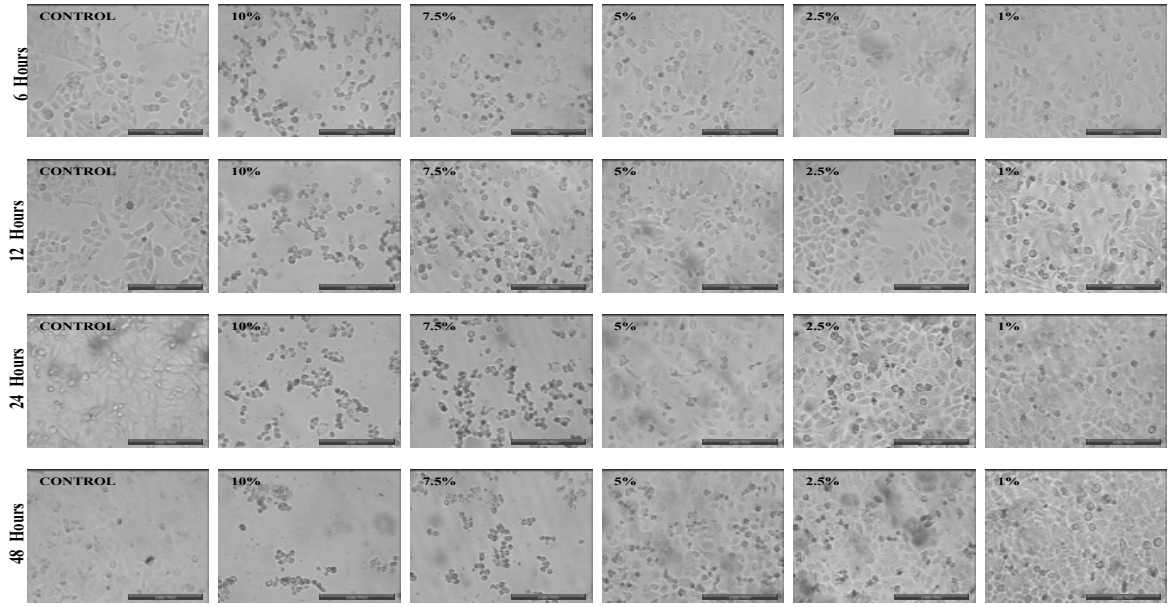

Figure 1. In-vitro Effect of Different Concentration of Penicillin at Different Time Interval in Cervical Cancer Cells (HeLa)

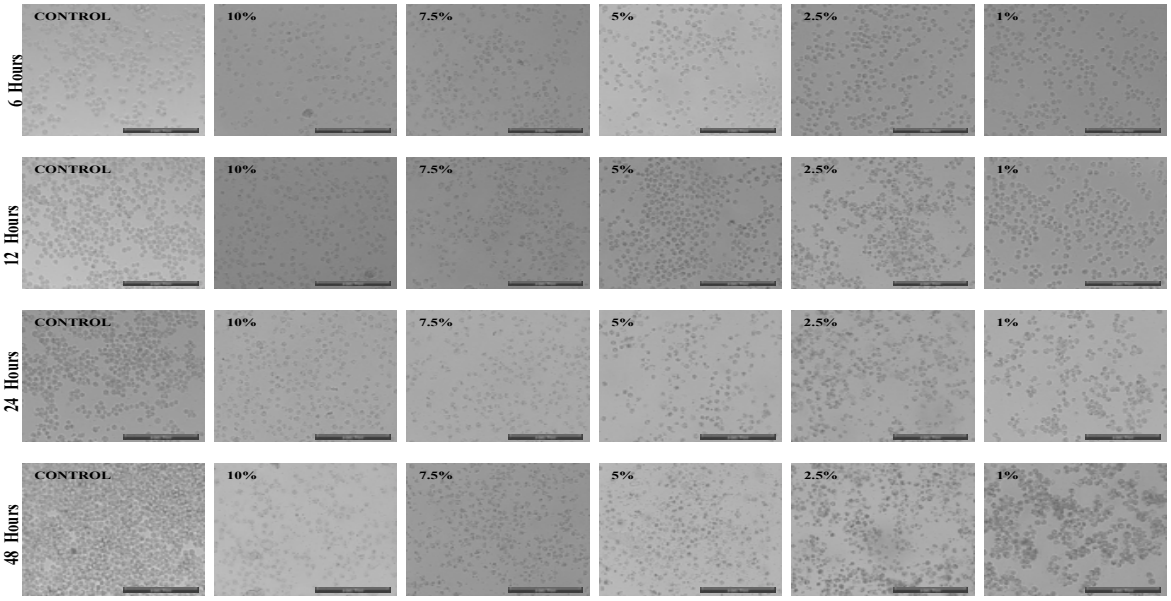

Figure 2. In-vitro Effect of Different Concentration of Penicillin at Different Time Interval in Leukemic Cancer Cells (K562)

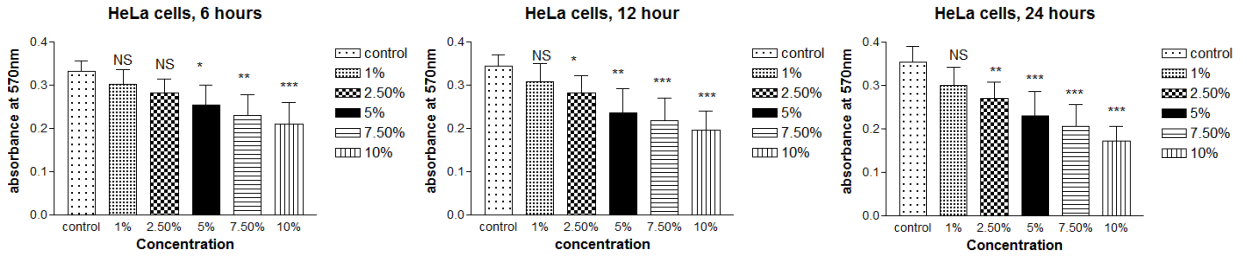

Figure 3. MTT Assay Graph of HeLa Cells after 6, 12 and 24 Hours of Penicillin Treatment

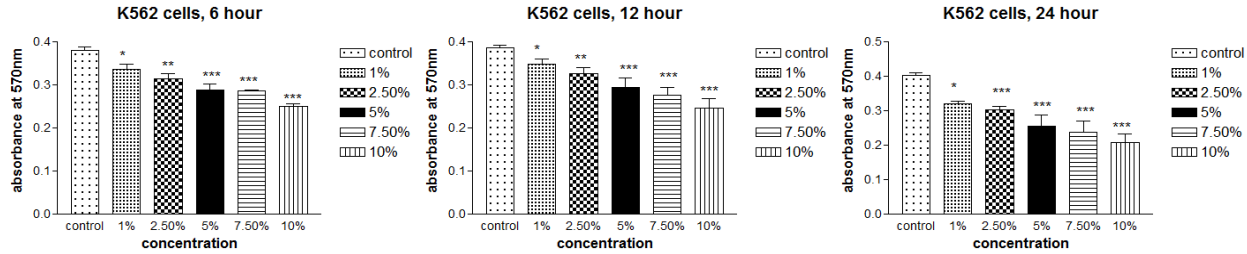

Figure 4. MTT Assay Graph of K562 Cells after 6, 12 and 24 Hours of Penicillin Treatment

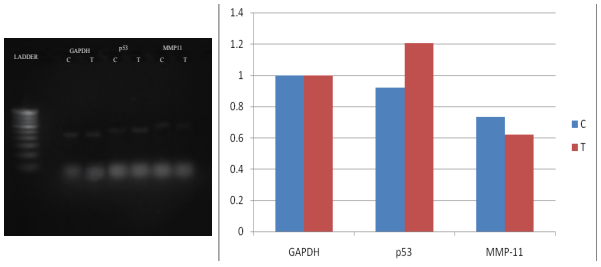

Figure 5. Gene Expression of GAPDH, p53 and MMP-11 in Control and 2.5% Treated HeLa Cells at Time Point of 24 Hours Using Polymerase Chain Reaction (PCR). Here, C=control, T=treated. In the graphical representation of gene expression, x-axis represents different genes and y-axis represents integrated density using Image J software

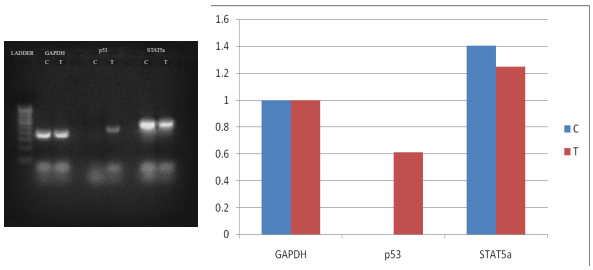

Figure 6. Gene Expression of GAPDH, p53 and STAT5A in Control and 5% Treated K562 Cells at Time Point of 48 Hours Using Polymerase Chain Reaction (PCR). Here, C=control, T=treated. In the graphical representation of gene expression, x-axis represents different genes and y-axis represents integrated density using Image J software

Gene expression of MMP11 in HeLa and STAT5a in K562 with respect to GAPDH and p53 genes

Gene expression analysis of HeLa and K562 at 2.5% and 5% concentration after 24 and 48 hours of penicillin treatment respectively showed down regulation of MMP11 (Figure 5) and STAT5a (Figure 6) in HeLa and K562 respectively and up regulation of p53 in treated cells in comparison to control cells. GAPDH was used as house keeping gene.

Discussion

Penicillin is known as the wonder drug because of its immense importance in the field of microbiology. In 1944, Corporal Ivor Cornman, performed an experiment on cells of a certain kind of mouse cancers growing in a test tube have succumbed to the drug. The penicillin damaged or killed cancer cells, left normal cells unharmed. It took three times the cancer-killing dose of penicillin to hurt the normal cells. When penicillin-treated cancer cells were transplanted to cancer-susceptible rats, none got cancer. Untreated cells gave cancer to all of them (Cornman, 1944). Apart from this, there is not a single work published where Penicillin is used against cancer cells. The purpose of the research was to find if Penicillin could be used as a potential treatment for cervical and leukemic cancer and also whether or not Penicillin plays any role in arresting the activity of cell division, proliferation and differentiation in HeLa and K562 cells. Matrix metalloproteinase's (MMPs) play a central role on the enhancement of tumor- induced angiogenesis, cell migration, proliferation, apoptosis and connective tissue degradation. MMPs -2 and -9 expression has been widely studied in cervical cancer (Basset et al., 1990). Expression of MMP11 in cervical precancerous lesions has been reported but not in histological normal cervix tissues (Rouyer et al., 1994). MMP11 expression was significantly higher in invasive carcinomas. Positive immune reaction was detected in the cytoplasm of cervix epithelium tumor cells, differing from other tumor tissues, where MMP11 expression is restricted to stromal cells that surround the neoplastic area (Kuhn et al., 2004). In this study, when HeLa cells were treated with penicillin, expression of MMP11 is down regulated. This shows that Penicillin might has significant effect on the degradation of the extracellular matrix where matrix metalloproteinase's (MMPs) play a central role. Signal transducers and activators of transcription (STATs) play important roles in numerous cellular events as for example differentiation, inflammation or immune response. Furthermore, constitutive STAT activation can be observed in a high number of tumors. The JAK/STAT pathway components may play an important role in the apoptosis mechanism of leukemic cells such as HL-60 and K-562 cells. It has been showed that K562 when treated with curcumin inhibited Jak2 mRNA expression and down regulated the activity of STAT5a (Blasius et al., 2006). Similarly, a significant decrease was seen in STAT5A mRNA relative expression level at 48 hours of Methylprednisolone treatment ($P<0.05$) in K-562 cells (Kaymaz et al., 2012). When penicillin treatment was given K562, STAT5A expression was down regulated. Other pathways may also be involved with a post-translational modification seen in the K562 cell line, with both up regulation and down regulation of protein expression levels of STAT5A. In case of Vero, cell where healthy and dividing 7.5% and 5% after 6 hours and 12 hours of treatment whereas in case of HeLa and K562 cells were dead in mentioned concentration. This proves that a minimum of double strength concentration is required to kill non-cancerous cells.

In conclusion, from above mentioned data it is clear that penicillin inhibits the growth of cancer cells even at lower concentration in comparison to non-cancerous cell. Further in vivo evaluation of penicillin is required to establish it as an anti-cancer agent.

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