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Combinatorial therapy of *Cynodon dactylon* and Metformin with Cisplatin in cervical cancer

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Abstract

Cisplatin based chemoradiation (CRT) is the standard treatment for cervical cancer, which controls tumor growth and improves the overall survival of patients. However, patients undergoing chemo-radiation show widespread toxicities which may be either early or late. There is a constant effort to improve cancer therapy and overcome current challenges in cervical cancer by developing a combinatorial drug therapy using phytocompounds.In the present study, we review the combinatorial therapy of *Cynodon dactylon* and metformin with cisplatin as an alternative therapy for cervical cancer. During frequent exposures to chemotherapy, patients develop resistance to cisplatin, leading to cytotoxicity and recurrence. The conjugate of biologically active moieties of natural products along with cisplatin will probably lead to development of a new therapy with improved drug efficacy and reduced toxicity. Therefore, *Cynodon dactylon* (Doob) is a natural source of antioxidants and metformin which is an antidiabetic and has anticancerous properties too. The combinatorial regimen of *Cynodon dactylon* and metformin along with cisplatin may increase the drug efficacy and reduce cisplatin-related toxicity. However, widespread research is required in this field for the mainstream application of this combinatorial therapy.

Keywords: Cisplatin; *Cynodon dactylon*; Metformin; Cervical cancer; Combinatorial therapy

Introduction

Cervical cancer is the third most common cancer among women worldwide. It is estimated that 6,04,127 new cases were diagnosed and 3,41,831deaths occurred due to cervical cancer annually[1]. In India, almost 1,23,907 women are diagnosed with cervical cancer and 77,348 die every year. Moreover, women or girls aged 15 years and older have a higher risk of developing the disease. In South Asia, the highest incidence of cervical cancer has been reported in India (18.0) as compared to Bangladesh (10.6), Sri Lanka (9.2), and Iran (2.3)[2].

Radiation therapy is known to be the treatment option for early-stage cervical cancer and chemotherapy helps to increase its effectiveness. Currently, CRT along with Cisplatin (cis-diammine-dichloro-platinum-II) is commonly used for cervical cancer as well as few other cancers[3-5].

This standard concomitant CRTis reported to have adverse effects like resistance and toxicity in patients[6]. Due to anti-cancerous properties of several phytocompounds, there has been a constant search for molecules that may reduce the adverse effects of existing therapies when given in combination[7]. Therefore, this review explores the possibility of using acombination of *Cynodon dactylon* and metformin along with cisplatin which might help to reduce its therapeutic drawbacks and result in better treatment outcomes in cervical cancer.

Research articles, reviews, case studies and books were selected from different online scientific and biomedical

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databases like PubMed, PubMed Central, Medline, EMBASE, International Pharmaceutical Abstracts and Web of Science.

Current clinical challenges in cervical cancer treatment

Cisplatin is an effective chemotherapeutic drug but has several side effects such as the development of resistance, cytotoxicity, neurotoxicity, gastro-intestinal and nephrotoxicity. During frequent exposures to chemotherapy, most patients are likely to develop resistance to Cisplatin, which further leads to diverse effects including cytotoxicity and probability of disease recurrence[4-5]. Cisplatin-induced toxicity is a complex pathway and has not been well understood. Studies suggested that the pathway affects membrane transport, nuclear and mitochondrial DNA damage, ionic homeostasis disruption, oxidative stress, chronic inflammation and apoptosis [8]. Although, most of cancer types show high responsiveness to cisplatin, the disease relapses in many patients due to chemoresistance. The mechanism behind cisplatin resistance includes cellular accumulation and biotransformation of cisplatin, liver detoxification, and increased responsiveness of the DNA repair system. Hence, a number of studies suggested that the efficacy of cisplatin can be increased by combination with other therapeutic drugs[5, 9-11]. A variety of phyto-compounds and plant extracts are known for their anti-cancerous, anti-inflammatory, antioxidant and chemo-preventive properties [9]. Therefore, the usage of such compounds in combination with cisplatin may reduce its toxicity and improve its sensitivity and efficacy[5, 7].

Mechanism of Cisplatinaction

Cisplatin induces DNA damage and cell death like other anti-cancer drugs [12]. Cisplatin forms intrastrand 1,2-d(GpG) and d(ApG) bifunctional DNA-cisplatin adducts that trigger the activation of the DNA damage response and apoptosis of cancer cells[13]. The recognition of DNA-cisplatin adducts contributes to the differential cytotoxicity level and disease severity. However, conformational changes between adducts may cause disruption in cellular activity by interacting with telomeres, free nucleobases before incorporation into the DNA making the cells more resistant[14].

HPV-mediated p53 degradation and p73 upregulation are associated with cisplatin resistance in cervical cancer. In cervical epithelial cells, cisplatin induces cytotoxicity by interfering with gene expression and/or DNA replication[15]. It controls the cancer progression *via* induction of apoptotic signal transduction and mitochondrial pathways, including calcium and death receptors[16]. Moreover, cisplatin passes the cell membrane *via* passive diffusion which is facilitated by various transporter proteins like CTR1, MRPs and OCTs. The impairment of cisplatin uptake, increase efflux and reduced accumulation has been associated with cisplatin resistance in cervical cancer cell lines[17]. However, internalized cisplatin binds with DNA and forms adducts and some of the molecules bind with nucleophilic species such as glutathione (GSH), methionine, metallothioneins (MTs),and other thiol-containing proteins[18]. This binding disturbs the redox, increases oxidative stress, reduces the bioavailability of cisplatin to bind with DNA and sensitivity for apoptotic signals in HeLa cell lines[19].

Cisplatin-DNA interaction (DNA adducts) has been identified as a DNA damage lesion which leads to activating predominantly the DNA repair system including base excision, mismatch repair and nucleotide excision repair[20]. The formation of Cisplatin-DNA adducts and activation of cell death is the major modes of anticancer action of cisplatin. Cisplatin-induced apoptosis takes place by activating multiple proteins including p53, Bcl2 family proteins, MAPK, and NF-κB. A down expression of p53, Bcl-2 and p-Bad was reported in cisplatin-resistant HeLa cells [8], while depleted activities of caspases including 3, 8 and 9 were found in SiHa cell lines[21]. Therefore, Cisplatin, directly and indirectly, is mediated by p53, caspases, calcium signaling, multidrug-resistance proteins, and reactive oxygen species (ROS) for inducing apoptosis(Figure 1).

Combinatorial therapy with cisplatin

Combinatorial therapy is a keystone treatment regimen for several diseases including cancers. However, cancer cells may become cisplatin-resistant during combinatorial chemotherapy[22]. There are various mechanisms of cisplatin resistance and widespread toxicities[23-25]. In order to overcome cisplatin resistance and resensitize the drug efficacy of cisplatin, it is required to develop alternative/combinatorial therapy or therapies which may prove to be more effective in defeating cancers[5, 22].

Kilic et al, (2015) [26] have found that cisplatin showed better efficacy in combination with epigallocatechin gallate in cervical cancer cell lines. This combination was critical for cell survival and cell death by regulating Akt/mTOR, and NF-kB signaling. It was shown that cisplatin and epigallocatechin gallate combination was less toxic and reduced cancer growth by decreasing cell survival signaling and enhancing cell death incisplatin-resistant cases. Combinatorial therapy of Cisplatin with epigallocatechin gallate in cervical cancer may be an option for severe cervical cancer[26]. It has been reported that curcumin inhibits HPV16 E6/E7 oncoproteins and positively influences the expression of CDKs,which lead to the arrest of cell cycle in G1-S phaseby inhibiting HDACs[27]. Furthermore, the suppression of PGP1 and MRP1 by curcumin aided in enhancing the therapeutic efficacy of cisplatin in cervical cancer [27].Currently available compounds/therapeutics have limited efficacy and/or safety concerns. Therefore, identifying novel bioactive phytocompounds will offer exciting possibilities for the development of promising anti-cancer therapies[8].

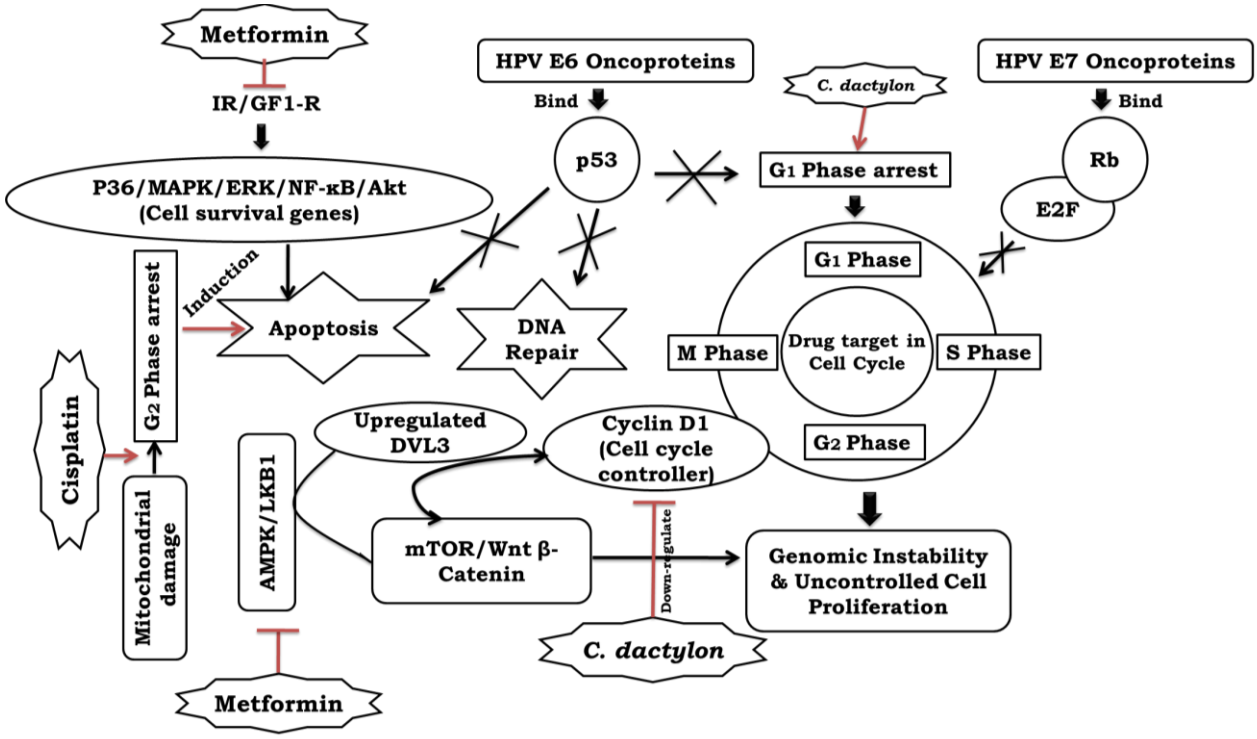

Figure 1: Overview of cervical cancer pathology and drug interventions.

Ayurved is a renowned, well-organized, preventive, and curative healthcare system using medicinal plants, herbs, shrubs and phytocompounds. Ayurvedic medicine originated >3000 years ago and is widely practiced in the eastern world. Recently, the global interest is to explore the therapeutic properties of plant-derived compounds or phytocompounds[28]. Desai et al, (2008) [8] has reported that several plant extracts and phytocompounds have shown a wide range of potential therapeutic properties including antimicrobial, antioxidant, anticancer, anti-inflammatory, analgesic and antipyretic[28-29]. Garlic (*Allium sativum*) is known for its medicinal value since ancient times which is the major source of garlic organosulfur compounds. Garlic extract containing sulfides stimulates apoptosis in cancer cells by upregulation of p53, p21 Bax, Fas, and downregulation of Bcl-2 family protein in pancreatic epithelial cell lines [30]. Similarly, curcumin is associated with inhibition of cervical cancer progression and increased efficacy of cisplatin by targeting cell death induction, ROS reduction *via* upregulation of p21 and p53, and downregulation of STAT3 and caspases. Therefore, natural medicinal compounds might improve the sensitivity of resistant anticancer drugs and can be a better drug candidate for the development of new therapeutic drugs for different diseases[5].

Studies have demonstrated that medicinal plants and their derivatives have been exploited to discover new potential therapeutics with less toxicity and side-effects[5, 29, 31-32]. Several medicinal plants and herbal ingredients have been experimentally proved to have anticancer effects[33]. A number of phytocompounds extracted from medicinal plants have shown to inhibit cell proliferation, activate apoptosis, inhibit angiogenesis and retard metastasis[32]. Various food and their derivatives showed preventive and potential therapeutic effects such as capsaicin (peppers)[34], curcumin (curcuma)[35], resveratrol (grapes)[36], lycopene (tomatoes)[37] and cinnamon essential oil[38]. α-Mangostin, a shrub derivative is well known for its anti-inflammatory, antioxidant, antitumorigenic and antibacterial properties as well as anti-proliferative activity[39].

The combinatorial effect of α-mangostin and cisplatin has been studied which showed distinct outcomes in a co-incubation and pre-incubation manner. The combined and simultaneous administration of α-mangostin and cisplatin caused a strong interaction and protection of the cancer cell[39-40]. On the contrary, the treatment with α-mangostin prior to cisplatin improved the therapeutic response exhibited by cisplatin[40]. This pre-incubation led to limited tumor growth by augmenting the cell doubling time without secondary effects (systemic damage and/or nephrotoxicity). This combinatorial treatment-induced apoptosis is *via* ROS production and cell cycle arrest[40]. Therefore, α-mangostin can be used as an adjuvant or supplementary agent along with cisplatin[6]. However, it is required for clinical trials to confirm the current efficacy.

Anticancerous potential of Metformin and Cisplatin

There is evidence that metformin shows anticancer activity and is associated with reduced cancer cell growth in diverse cancers[41-44]. The enzyme liver kinase B1 (LKB1) expression determines the therapeutic efficacy of metformin in the treatment of cervical cancer by the activation of LKB1/AMPK signaling[45-46]. Siddik (2003) [47] reported that metformin protects against cisplatin cytotoxicity and reduces oral squamous cell carcinoma cell proliferation by arresting cells at G0/G1 phase. Metformin not only reduces cell proliferation but also protects against mitomycin C, a DNA-crosslinker[48, 49]. Metformin decreases cisplatin-DNA adduct formation by inducing glycolysis and intracellular NAD(P)H levels with reduced intracellular thiols[50]. Furthermore, it is reported that metformin reduces blood glucose and increases sensitivity of insulin by regulating cancer-promoting IGF-1 signaling and blocking the epithelial-mesenchymal transition [44].

However, the combination of metformin along with cisplatin (high dose of metformin, and lower dose of cisplatin) is more effective for apoptosis and cancer growth suppression in cervical cancer cell lines. This combination reduces the dose-dependent toxicity and controls the energy metabolism by induction of AMP-activated AMPK and inhibition of mTOR[51]. Similarly, the combinatorial effect of metformin and caffeic acid along with cisplatin resulted in a reprogrammed cell cycle, apoptosis and resensitization of SiHa cervical cancer cells [52]. Interestingly, it has been reported that metformin is not only limited to anticancer effects as seen in cervical cancer but also suppresses growth in case of thyroid cancer by increasing the p53 level and activates ERK-mediated apoptosis in breast cancer[53]. Likewise, the co-administration of metformin and carboplatin (an analog of cisplatin) showed increased mitochondrial-associated cell death in cervical cancer cells and enhanced the sensitivity of cisplatin/analogs to cancer cells by decreasing mitochondrial membrane potential[54]. Deng et al, (2017) [55] also found similar results that metformin alone with cisplatin promoted cell death and inhibited cancer growth by modulating the expression of VEGF, VEGFR2, ERK1/2-signaling in ovarian cancer cells[55].

Anticancerous potential of *Cynodon dactylon*

Cynodon dactylon (Poaceae), a perennial grass, is one of the most commonly occurring weeds in India, it is known as *dhub*, *doob*, or *harialil*, *durva*or *haritali* (Sanskrit). It is cultivated throughout the tropics and subtropics. The whole herb and its rootstalk are used for medicinal use[56]. It possesses a variety of medicinal values such as antiviral, antimicrobial[57], anti-inflammatory[58], immunomodulatory [59], and has significant application in treating dysentery, dropsy, hypolipidemia and hypoglycemia[60]. Twenty-two bioactive phytocompounds were reported in the extract of *C. dactylon*[61]. It has been reported that *C. dactylon*[62] has anticancerous activities in different types of cancers similar to metformin[63].

Kanimozhi et al, (2013) [64] reported that the leaf extract of *C. dactylon* controlled tumor growth by induction of apoptosis and cell death in colorectal carcinoma. The extracellular/intracellular regulation of cyclin D1 is required in cell cycle and related pathways[65]. The phosphorylation of cyclin D1 leads to the activation of a transcription factor, E2F which moves the cells from G1 to S phase. Hence, cyclin D1 induces angiogenesis, inhibits apoptosis, and promotes abnormal cell growth during malignancy[66]. It was demonstrated that leaf extract of *C. dactylon* down-regulates cyclin D1, phosphorylates Rb protein, arrests cell cycle in G1 phase and induces cell death in HNK-1 cells[62]. Several studies reported that it inhibited the growth of colorectal, liver, colon and breast cancers[57, 64, 67].

Conclusion

Phytochemicals possess anticancer properties which can serve as potential pilot compounds in drug designing. Targeting cancer cells with combination(s) of chemotherapeutics and phytochemical(s) may provide helpful insight for the development of novel therapeutic strategies against cervical cancer. A synergistic approach may give better and more effective medication as compared to current therapies including radiation and chemotherapy. Developing new cancer regimens may overcome tumor burden in the population. Thus, a combinatorial therapy of *Cynodon dactylon* and Metformin with Cisplatin may lead to the design of more efficient therapeutic strategies against cervical cancer with reduced side effects. However, widespread research is required to evaluate the efficacy of combinatorial therapy and its application in mainstream treatment regimens.

Therefore, future *in vivo* and *in vitro* studies are warranted to investigate the effects of combinatorial therapy of *Cynodon dactylon* and metformin with cisplatin in cervical cancer. This therapy may become a unique chemotherapeutic regimen and help to overcome cisplatin resistance and improve drug efficacy.

No conflict of interest.

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Ethical approval

No ethical approval required.

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