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Anti-cancer Efficacy and Mechanisms of Usnic Acid

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ABSTRACT

Cancer is the second deadly disease after cardiovascular diseases. The presently available therapeutic strategies of cancer are insufficient for the cure and betterment of cancer patients. Herein, we have reviewed the therapeutic potential of a lichenic secondary metabolite, the usnic acid, with special emphasis on its anti-cancer efficacy and associated mechanisms. Usnic acid has various biological activities that have been explored and it is utilized by humans from ancient times throughout the globe. A summary of the anti-cancer properties of usnic acid in different cancer types and models is presented. Usnic acid has been shown to inhibit the cancer cell proliferation *via* suppressing the clonogenic potential, decreasing the expression of PCNA (proliferating cell nuclear antigen), and activation of the tumor suppressor genes. Primarily, usnic acid induces reactive oxygen species (ROS) in cancer cells that lead to DNA damage, further causing the activation of deoxyribonucleic acid (DNA) damage response that finally initiates the apoptotic pathways. The ROS induction was found to activate the JNK pathway. It also depolarizes the mitochondrial membrane, induces the release of cytochrome-c, and activates the caspase cascade and cleavage of PARP that ultimately results in programmed cell death of cancer cells. Overall, this lichen metabolite has a strong efficacy against cancer cells, which warrants further investigation for its potential clinical uses.

INTRODUCTION

Usnic acid is found uniquely in various lichen species. It is bitter and has a yellowish cortical texture. Lichens are a consortium of two nutritionally specialized organisms i.e. green alga or cyanobacteria and fungi, where both are benefited [1]. There are several biologically active properties found in lichens. Therefore the lichens are explored and utilized more extensively by mankind for various purposes such as floral decorations, dyeing, dietary, medicinal, and, most importantly for pollution monitoring [2]. Lichens produce hundreds of secondary metabolites but the most exclusive metabolites may belong to three chemical classes, namely, depsidones, depsides, and dibenzofurans [2]. Usnic acid is a dibenzofuran derivative, which is widely distributed in several classes (and families) of lichens such as *Usnea* (Usneaceae), *Cladonia* (Cladoniaceae), *Alectoria* (Alectoriaceae), *Parmelia* (Parmeliaceae), *Ramalina* (Ramalinaceae), *Ervinia* and various other lichens [3]. The maximum yield of ~6% (w/w) usnic acid is reported in *Alectoria* (Alectoriaceae) species [3]. If we look at its chemical structure, it has two enantiomeric forms, namely (-)-usnic acid and (+)-usnic acid [4].

The practice of using usnic acid-containing lichens for various therapeutic purposes is ancient and popular throughout the globe. The consecutive studies have recognized the potential of usnic acid in various biological activities such as anti-proliferative, anti-oxidant, UV protective, anti-microbial, anti-protozoal, anti-viral, larvicidal, anti-inflammatory, and healing [2, 5]. The crude extract of *Usnea* species is used in fever control and pain relief [6] and *Cladonia* species for the treatment of pulmonary tuberculosis in various parts of Asia, Europe, and Africa [7]. *Ramalina thrausta* have been used externally for the treatment of various skin illness, wounds, athlete's foot, and also orally for the treatment of toothache and throat sore [7]. In the present article, we have reviewed some of the

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important activities of usnic acid with special emphasis on anti-cancer potential and associated mechanisms.

ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory property of usnic acid was recognized when an extract of *Roccella montagnei* was tested on rats, where it shows a dose-dependent anti-inflammatory response in both acute and chronic models [8]. It is also reported usnic acid attenuate inflammatory responses and induce oxidative stress in acute lung injury of mice induced by LPS. Further usnic acid suppressed the expression of important inflammatory response molecules such as interleukin-6, interleukin-8, tumor necrosis factor (TNF-α), and macrophage inflammatory protein-2 (MIP-2). Simultaneously, it upgraded the interleukin-10 expression in bronchoalveolar lavage fluid (BALF) [9].

In an *in vitro* study, Huang *et al*., treated LPS stimulated RAW 264.7 cells with usnic acid and observed a downregulated expression of pro-inflammatory mediators such as COX-2 (cyclooxygenase-2), iNOS (inducible nitric oxide synthase), IL-6, and IL-1β. The gene expression of TNF-α and COX-2 was found suppressed through decreased activation of NF-κB (nuclear factor-κB) and increased expression of anti-inflammatory cytokine IL-6 and HO-1 (heme oxygenase-1) [10].

WOUND REPAIRING ACTIVITY

Currently, various cosmetic and pharmaceutical preparations include an extract of *U. barbata* as a source of usnic acid. Since, repairing of the wound is a major concern in numerous pathologies such as burns, chronic wounds, scars, vaginal lesions, topical otitis, and mouth infections hence, there is a need for new chemical entities that can reduce the use of hazardous and toxic chemicals. Burno *et al*. synthesized usnic acid enamine and evaluated their effects on wounds using *in vitro* and *in vivo* models. They attributed substantial wound healing properties in the derivatives of usnic acid. The results were quite consistent in showing the highest healing performance with the lowest cytotoxicity *in vitro* and *in vivo* models [11].

In another study, Nunes *et al*., showed that usnic acid enhanced the burn healing in rats when a collagen-based film is loaded with usnic acid. The use of usnic acid modulated epithelization, collagen formation, and inflammatory responses in this process [12, 13]. In a recent study, usnic acid has been shown to improve the bactericidal property of polyaniline and potentiate the anti-biofilm activity of lichen derivatives when usnic acid is doped on undoped polyaniline. The resulting material, when installed on polyurethane foam it boosted their wound dressing applicability [14]. Further, a study was performed to investigate the microscopic and macroscopic changes in dermal injury after the use of sodium usnic acid on it. Sodium usnic acid increased the rate of wound healing and decreased the re-epithelialization time, epidermal keratinization time, and formation of well-organized collagen bands. Histological studies showed that usnic sodium acid reduced the inflammatory cells and increased the granulation tissue, fibroblast proliferation, and vascular regeneration [15].

UV-PROTECTION ACTIVITY

The ozone depletion over a recent decade is a major environmental threat that could be addressed on an urgent basis. Nowadays, the earth's surface is getting extra ultraviolet radiation exposure which is damaging our skin and causes various diseases such as hyperpigmentation, edema, erythema, photoaging, and skin cancer [16, 17]. The present preventive strategies include UV blockers, which are expected to act like UVA-UVB filters and to have anti-oxidant and photo-stable properties without any dermal toxicity. To achieve a high index of protection, the combination of organic and mineral filters as an essential component is required for a guaranteed sunscreen [18]. The UV absorption properties in lichens are mainly due to the presence of aromatic compounds with high conjugated bonds, which shields its photosynthetic pigment from photo-oxidative radiations [19]. Usnic acid is described as a good UVB absorbent with an advantage of no dissipation of radiation as fluorescence, but a moderate *in vitro* sun protection factor (SPF) value [20].

Moreover, various studies have shown that usnic acid has anti-microbial activity. This activity of usnic acid is due to its potential to inhibit the DNA and RNA synthesis, which led to hinder the growth of gram-positive bacteria [2, 21, 22]. However, its anti-viral effect is due to the inhibition of RNA transcription [23].

CANCER

Cancer is the second leading cause of death next to cardiovascular disease worldwide (WHO 2018). The global burden of cancer is significantly augmented in terms of incidence and mortality each year. This augmentation is explained by upgraded life expectancy that offers extra time for clinical indicators of cancer. The present 18.1 million cases of cancer are expected to increase to 29.4 million in 2040. Apart from non-melanoma skin cancer, the estimated death caused by cancer in the year 2018 was 9.5 million that is expected to cross 43 million deaths in 5-year prevalence worldwide [24].

Cancer may refer to a combination of diseases in which the cells divide abnormally and infiltrate into various parts of the body and destroy normal cells and organs. All abnormal growth of cells in the body, which referred to a tumor is not cancer. Out of two types of tumors, i.e., benign and malignant, the malignant tumors are cancerous that have the potential to mature, permeate to different parts of the body and initiate the sufferings to the patients. The tumor formation is recognized as a multistep process that may include genetic and epigenetic alterations that transform the normal cells into premalignant to highly malignant offshoots.

There are several therapeutic approaches, including chemotherapy, surgery, radiotherapy, immunotherapy, hormone therapy, and targeted therapy, that have been used depending on the types and stages of cancer. Still, cancer is one of the leading challenges for our society because of poor survival rates and compromised quality of life among patients. In such a scenario, the naturally occurring chemicals may offer an alternative to deal with cancer.

USNIC ACID AND CANCER

Usnic acid as an antitumor agent was recognized in the year of 1975. Initially, it was detected that the *Cladonia leptoclada* (Cladoniaceae) extract has significant tumor inhibitory activity when applied in Lewis lung carcinoma of mice. Further, researchers fractionated the extract and found (-)-usnic acid as the principal constituent having this property [25]. Further, in another study, it was shown that (-)-usnic acid has moderate activity against both murine P388 leukemia test system and in cultured L1210 cells [26]. Moreover, many derivatives of the parent compound were prepared by disrupting the intramolecular bonds, despite increased hydrophilicity; none of the synthesized derivatives were more effective than the mother compound. From this study, β-triketone moiety was detected as the parent compound played a vital role in maintaining its activity [26].

(+)-usnic acid, when exposed to endometrial carcinoma HEC-50, Ishikawa, and leukemic K-562 cell lines for 21 hours, the cell count was found to be reduced, which was thought to be due to its antimitotic activity. The effect was significantly increased when the exposure time was increased to 46 hours, this indicated the importance of the time of exposure that ultimately modulated the mitotic activity of the cells [27]. Usnic acid has inhibited cell proliferation of p53 variant cell lines of breast cancer such as MDA-MB-231 with non-functional p53, MCF-7 with wild type p53, and also lung cancer cell line H1299 with null p53. The non-genotoxic property and action against cancer cells irrespective of p53 expression made usnic acid a novel candidate for cancer therapy [28].

In a recent study, we have exposed the gastric cancer AGS cells with usnic acid and noted their effect on cell proliferation. Usnic acid was found to inhibit the cell proliferation of AGS cells via modulating the expression of tumor suppressor genes. The up-regulated PTEN and the down-regulated PCNA expression indicated the relevance of this study in highlighting the usnic acid as a probable cancer-targeting agent in gastric cancer cells [29]. Similarly, the non-cytotoxic concentration of usnic acid has shown to suppress the replicative potential and hence reduced the clonal expansion of gastric and lung cancer cells [30, 31]. The summary of the above study is presented in Figure 1.

Further, the role of usnic acid in apoptosis induction is established in human breast cancer and gastric cancer cells [5]. Various other studies have also supported the notion that usnic acid has antitumor potential, as it inhibits proliferation, metastasis, and angiogenesis through different signaling pathways in different types of tumor cells [32]. To explore the efficacy and associated mechanism in human lung carcinoma cell, Singh *et al*. treated A549 cells with a clinically achievable concentration of usnic acid. They reported that (+)-usnic acid have anti-proliferative and growth inhibitory effect along with suppression of clonal growth and a G0/G1 phase cell cycle arrest. Usnic acid modulated the cell cycle regulators such as CDK4 and 6, P21/Cip1 and cyclin D1 in A549 cells. It has also been illustrated that mitochondrial membrane depolarization and cleavage of PARP are the mediator of apoptosis in A549 cells [31].

In another study, a time and dose dependent Reactive Oxygen Species (ROS) induction was reported in MCF-7 breast cancer cells. Usnic acid-induced ROS triggered the activation of JNK, c-jun, and ERK. They also found a rapid loss of mitochondrial membrane potential, increase in Bax/Bcl-2 ratio, and release of cytochrome-c, activation

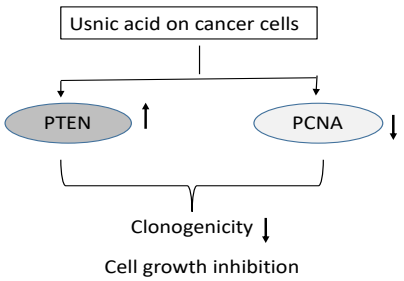

Figure 1: Cell growth inhibitory effects of usnic acid on cancer cells. Usnic acid increased the expression of PTEN and decreased the expression of PCNA, which further inhibits the cell growth and clonal expansion of cancer cells

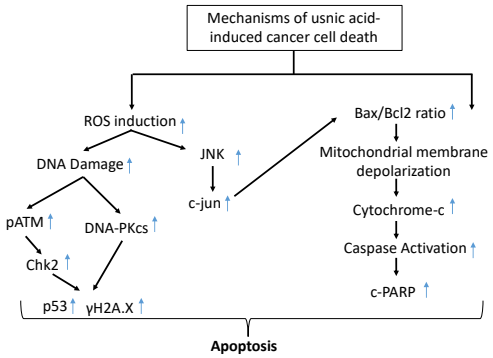

Figure 2: Mechanisms of usnic acid-induced apoptotic cancer cell death. Usnic acid induces ROS generation in cancer cells. The ROS induction causes severe DNA damage and spontaneous DNA damage response activates the ATM, Chk2, p53, DNA-PKcs, γ-H2A.X, and JNK and ultimately leads to apoptosis. The activation of JNK by ROS Depolarizes the mitochondrial membrane potential and activates cascade leading to apoptosis.

of caspase-3 and cleavage of PARP in usnic acid-treated cells. Moreover, usnic acid was found to suppress the tumor weight and size by inducing apoptosis in tumor cells without obvious toxic side effects in an MCF-7 xenograft mouse model. The efficacy of usnic acid was shown similar to cyclophosphamide (CTX), the first-line drug for breast cancer therapy [5].

In the further studies, we have reported the molecular alterations in gastric cancer AGS and SNU-1 cells with the treatment of usnic acid. The treatment of usnic acid caused apoptosis in AGS cells which were regulated by the dissipated mitochondrial membrane and cleavage of PARP. The increased Bax to Bcl-2 ratio favored the loss of mitochondrial membrane potential and led to apoptosis. Moreover, usnic acid increased the concentration and time-dependent generation of ROS which was found to be involved in DNA damage in gastric cancer cells. The DNA damage activated response of AGS cells increased the expression of several damage repair proteins such as p53, Chk-2, pATM, γH2A.X, and DNA-PKcs which later promote apoptosis. However, a similar concentration of usnic acid did not inhibit the growth of primary gastric cells of the mouse as well as non-neoplastic, HaCaT, and HEK-293 cells [30]. However, further validation of the usnic acid *in vivo* model to develop as a potential anti-cancer agent is required. The mechanism of usnic acid-induced cell death is summarized in Figure 2.

Furthermore, Yang *et al*. have shown that usnic acid acts as a suppressor of cancer cell motility. They found that usnic acid suppresses lung cancer cell invasion and migration by decreasing the KITENIN-mediated AP-1 activity and β-catenin-mediated TOP FLASH activity in a dose-dependent manner. Usnic acid suppresses the downstream target genes of β-catenin/LEF and c-jun/AP-1 at mRNA levels such as Cyclin D1, CD44, and c-myc. Rec1 and RhoA activity was also found to be decreased. These studies suggested the anti-metastatic potential of usnic acid in cancer cells [33]. Additionally, a few studies have suggested that usnic acid can induce hepatotoxicity if consumed frequently [34, 35]. This should be taken into consideration for its pharmacokinetic analysis.

CONCLUSIONS

The relation between cancer and inflammation is well established. Chronic inflammation can lead to cancer. The anti-inflammatory effects of usnic acid support its cancer-preventive properties. However, the detailed mechanism of the mode of action of usnic acid in association with inflammation and cancer is still elusive. Further, usnic acid is reported to have wound repairing properties after its external use. Cancer is described by a variety of cellular phenotypes including a wound that never heals. Nevertheless, the application of usnic acid can heal a non-cancerous wound; however, its effect on the healing of cancerous wound requires more studies.

Despite the lack of clinical studies with usnic acid, various scientific investigations have highlighted its anti-cancer properties in cell culture models. Now, the mechanisms of the action expressed by usnic acid in cancer cells are partially uncovered. The role of ROS induction by usnic acid has been suggested to be linked with apoptosis initiation in various cancer cells. DNA damage, activation of DNA damage response, mitochondrial membrane depolarization are involved in the launch of apoptosis cascade. Further, usnic acid has the potential to also inhibit metastasis in some cancer cells. However, a detailed mechanism is yet to be explored.

A few studies suggested that usnic acid has hepatotoxic effects at higher and chronic doses. However, these limitations can be eradicated by their mindful uses and application. The solubility of usnic acid was also proven a hindrance in its medicinal uses, but these limitations can also be overcome by making its salt with other hydrophilic compounds. Moreover, further studies are necessary to explore other associated mechanisms of usnic acid at the molecular level, and determining the safe toxicological parameters for its effective uses in therapeutics of cancer.

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