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Research article

Reserpine subdued non-small cell lung cancer cells via ROS-mediated apoptosis

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ABSTRACT

Non-small cell lung cancer (NSCLC) is the prevailing kind of lung cancer. Molecular target has exceptionally improved the treatment viability of lung cancer, however, new difficulties have developed, for example, drug resistance and cancer repeat. Along these lines, new chemotherapeutic operators and treatment techniques are earnestly required. Reserpine, a natural indole alkaloid, is the fundamental dynamic piece of Indian restorative plant which has been appeared to show ground-breaking hostile to cancer action in specific kinds of cancer; be that as it may, its movement in drug-safe lung cancer has never been tended to. In this investigation, we utilized 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) measure and 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) test to examine reserpine anticancer movement. The cytotoxicity of reserpine in NSCLC cell lines was profoundly investigated. Reserpine displayed specific cytotoxicity among NSCLC at 15, 25 and 35 µM concentrations. Reserpine essentially expanded the action of ROS, which are profoundly expanded apoptosis. Improved ROS, altogether initiated customized cell passing in human NSCLC cells. The upgrade of ROS age in drug-safe NSCLC cells prompts impedance of development and acceptance of apoptosis. In this way, reserpine-prompted cell apoptosis is firmly connected with ROS rise in the cells. These discoveries show that reserpine can be a powerful mix of treating drug-safe NSCLC.

INTRODUCTION

Lung cancer is the most widely recognized harmful tumour and the main source of cancer-related demise on the planet (Lemjabbar-Alaoui et al., 2015). Non-small-cell lung cancer (NSCLC) represents around a wide range of lung cancer and incorporates adenocarcinoma, squamous cell carcinoma and enormous cell carcinomas (Zappa and Mousa, 2016). More than 1.5 million new instances of lung cancer are analyzed each year, around 80% of which are non-small cell lung cancer (NSCLC) (Dela Cruz et al., 2011). The horribleness is quickly expanding chiefly because of environmental contamination and undesirable ways of life. The endurance rate remains generally low significantly after a medical procedure, chemotherapy or radiotherapy. More than 90% of the cancer-related mortality can be credited to metastasis (Ozlu and Bulbul, 2005). Such hindrances make it hard to viably treat lung cancers. An earnest clinical need exists for novel medicines either focusing on or forestalling metastasis, particularly in beginning phase cancer patients. One of each three cancer-related passings is owing to lung cancer and has no improvement throughout the last around 30 years (Pinsky et al., 2015). Hence, there is an earnest need to recognize biomarkers and medication focuses on early conclusion and treatment for NSCLC.

At present, chemotherapy assumes an essential job in far-reaching NSCLC treatment. Cisplatin-based medications are the most broadly utilized chemotherapy medication for NSCLC. Phytochemicals of restorative qualities offer promising choices for the improvement of successful procedures for the avoidance of tumour cell movement, attack and metastasis. Also, some chemo-drugs focusing at microtubule including docetaxel, cabazitaxel, epothilones and vinorelbine have been utilized for NSCLC treatment (Davies et al., 2003).

Reserpine (Fig. 1), an indole alkaloid found in *Rauvolfia serpentina*, confined from assorted

therapeutic plants, displayed cytotoxic exercises against different cancer cell lines by actuating autophagy, necroptosis and apoptosis affected by different proteins engaged with the apoptotic pathway. Three mixes, to be specific vinblastine, vincristine, and vindesine, were separated from *Catharanthus roseus* and were broadly concentrated as anticancer operators (Moudi et al., 2013). Vinca alkaloids repressed multiplication of cells by changing the elements of tubulin expansion and misfortune toward the finish of microtubules of the mitotic shaft (Ngan et al., 2001). Vincristine and vinblastine are commonly utilized in blend with other chemotherapeutic specialists for the treatment of lymphomas, leukaemias, bosom, lung cancer, progressed testicular cancer, and Kaposi's sarcoma (Falzone et al., 2018). Vinblastine, an autophagy development inhibitor, in a mix with nanoliposomal C6-ceramide (autophagy inducer) synergistically, improved the apoptotic cell passing in HepG2 (human hepatocarcinoma) and LS174T (human colon) cell lines by rising the autophagic vacuole gathering and diminishing the autophagy development of the phones. In any case, the anticancer movement of reserpine on lung cancer remains indistinguishable. In this assessment, we intended to investigate the movement of reserpine on NSCLC via ROS.

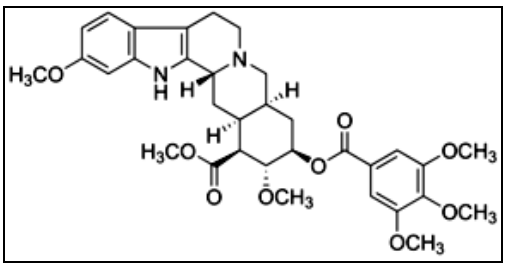

Fig. 1. Chemical structure of reserpine

MATERIALS AND METHODS

Chemicals and reagents

Dulbecco's Modified Eagles Medium (DMEM), Phosphate Buffered Saline (PBS), fetal bovine serum (FBS), 0.25% trypsin EDTA, antibiotics (penicillin, streptomycin), dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 2,7-diacetyl dichloro-fluorescein (DCFH-DA), Ethidium Bromide (EtBr), Rhodamine 123, Acridine Orange (AO), Hoechst 33342 stain were obtained from Hi-media Lab Ltd., Mumbai, India. Reserpine was kindly gifted by Dr B.A.A. Abdul, Faculty of Applied Science, Ton Duc Thang University, Ho Chi Minh, Vietnam.

Cell culture and treatment

The NSCLC human lung cell line was obtained from the National Centre for Cell Sciences, Pune, India. Cells were seeded into plates, flasks, or dishes in Dulbecco's Minimal Essential medium supplemented with 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, anti-biotic solution (Sigma) in a humid atmosphere of 5% CO_2 and 95% air at 37 °C. The media were changed on alternative days.

Measurement of cell viability by MTT assay

MTT assay was carried out to determine the cytoprotective effect of indole alkaloid on cell proliferation in NSCLC cells. Briefly, cells (5×10^4) were seeded into 96-well tissue culture plates and incubated under the above-mentioned conditions. After 24 h of incubation, cells were treated without or with 1-100 μM of reserpine. After 24 h of incubation 20 μL of MTT (5000 μg/mL) was added into the culture medium. Four hours later, the medium containing MTT was aspirated and replaced by solubilization solution (DMSO) for 30 min. Following this incubation; the absorbance was measured in an ELISA reader at 570 nm.

Determination of intracellular ROS levels

Intracellular ROS level was measured using a non-fluorescent probe, 2,7-diacetyl dichloro-fluorescein (DCFH-DA). This probe can penetrate into the intracellular matrix of cells where it is oxidized by ROS to fluorescent dichlorofluorescein (DCF). Indole alkaloid treated NSCLC cells were seeded in 6 well plates (2×10^6cells/well) and incubated with 10 M DCFH-DA for 30 min at 37 °C. Fluorescent measurements were made with excitation and emission filters were set at 485 ± 10 nm and 530 ± 12.5 nm, respectively (Shimadzu RF-5301 PC spectrofluorometer). The cells were also observed under a fluorescence microscope using blue filter (450-490 nm) (Nikon, Eclipse TS100, Japan).

Statistical analysis

Data are expressed as mean ± standard error (SE) for a minimum of three independent determinations in triplicate for every experimental point. Data were analyzed using SPSS Statistics software. For all the measurements, one-way analysis of variance followed by Duncan's new multiple range tests ($p \leq 0.05$) was used to assess the statistical significance of the difference between control and treated groups.

RESULTS

Effect of indole alkaloid on cell cytotoxicity

The cytotoxic effect of indole alkaloid on NSCLC cells was determined by MTT assay (Table 1). Cells were treated with different concentrations of indole alkaloid (1-100 μM) for 24 h incubation, which revealed a dose-dependent inhibition of cell proliferation. Maximum cell death was observed at 70 μM concentrations. Hence, the IC_{50} of reserpine

for NSCLC cells 35 µM apparent from growth inhibition curve. We selected 15, 25 and 35 µM doses of reserpine for further studies.

Effect on the generation of intracellular ROS

The intracellular ROS generation was measured by DCFH-DA staining. Fig. 2 illustrates the levels of ROS generation in control and indole alkaloid treated cells. NSCLC cells were treated with different concentration of reserpine (15, 25 and 35 µM) shows significantly increased levels of ROS generation which indicating extreme green fluorescence intensity as compared to untreated control cells.

Table 1. The inhibitory concentration of reserpine against NSCLC cells using MTT assay

Replication	Control	15 µg/ml	25 µg/ml	35 µg/ml
1	47.8 ± 4.9	35.4 ± 3.1	26.5 ± 2.9	17.5 ± 1.14
2	45.2 ± 4.5	35.8 ± 3.4	25.5 ± 2.4	14.8 ± 1.09
3	49.0 ± 4.8	37.0 ± 3.2	24.2 ± 2.1	15.0 ± 1.11

Data are expressed as Mean ± SE.

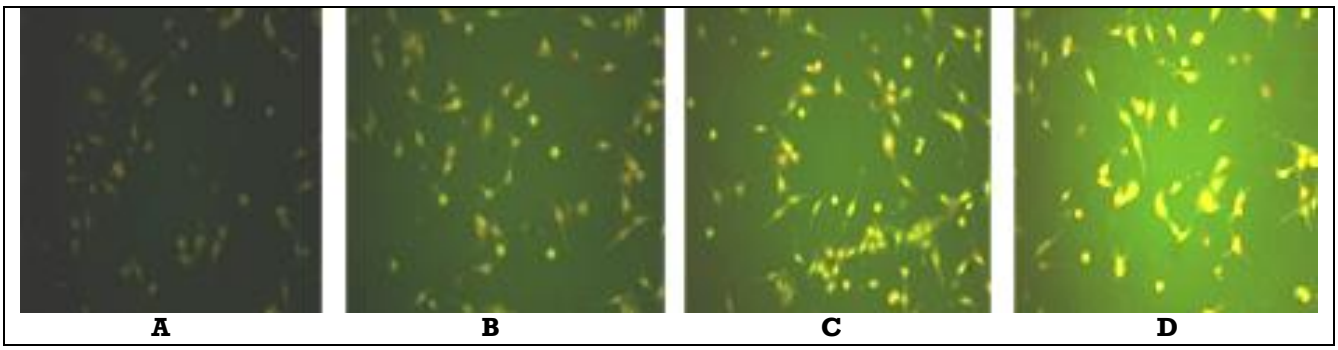

Fig. 2. The effect of reserpine on intracellular ROS generation. Photo micrographic image of A: Control cell shows weak fluorescence; B: Treatment with 15 µg/ml shows mild fluorescence; C: Treatment with 25 µg/ml shows moderate fluorescence; D: Treatment with 35 µg/ml shows enhanced DCF fluorescence indicating increased ROS generation.

DISCUSSION

The recognizable proof of atomic procedures associated with cancer improvement and guess has opened up roads for focused treatments, which has made treatment more tumour-explicit and less harmful. Nonetheless, most molecularly focused on treatment is associated with cancer tranquillize protection from a specific degree, and one notable model is ROS (Cui et al., 2018). Consequently, difficulties, for example, tranquillize obstruction stayed uncertain in focused treatments (Groenendi and Bernards, 2014). Given that a dominant part of natural chemotherapeutic operators is naturally happening mixes, it is promising to distinguish and create novel medications from natural assets, for example, alkaloid, that can explicitly assault ROS-subordinate tumour cells (Sznarkowska et al., 2017).

Different examination recognized reserpine, which can specifically restrain lung cancer cells by over initiation of ROS, bringing about exorbitant increments in ROS and particular oxidation (Mishra et al., 2018), prompting over-oxidation actuation and oxidative pressure. Thus, under hypoxia condition which will strikingly improve cell ROS level, cells experienced apoptosis (Simon et al., 2000).

This data gave a totally new skyline to procedures to treat safe NSCLC. Even though this novel treatment procedure is promising, it is hard to additionally screen ROS activators as the kinase enactment. Likewise, screening kinase activators is more troublesome than kinase inhibitors; in this manner, the improvement of novel chemical inhibitors instead of activators remains the key methodology in the advancement of the little atom-based chemotherapeutic operators.

In this examination, we further researched another potential reserpine that expansion ROS in safe NSCLC by means of restraint of cell proliferation. Natural reserpine has different organic exercises, remembering its utilization for tumour treatment, for example, osteosarcoma, leukaemia, bosom cancer, and carcinoma of the salivary organ (Xu et al., 2020; Rajesh et al., 2015; Mukhopadhyay et al., 1981). In this, we exhibit reserpine applied potential enemy of tumour action in lung cancer cells.

Apoptosis is a vital homeostatic instrument that adjusts cell division and cell demise to keep up the uncontrolled proliferation of cancer cells (Askew et al., 2017). In the current examination, reserpine essentially incited apoptosis cell demise in NSCLC cells, and debasement, which lead to enactment and resulting harm of DNA. Our outcomes

indicated that reserpine prompted ROS cell demise in a fixation subordinate way.

Moreover, our outcomes demonstrated that the component of the counter tumour property of reserpine included the enlistment of ROS (Zhu et al., 2015). We further affirmed that reserpine successfully initiated cell debasement, and hence smothered oncogenic flagging pathway, which additionally added to hostile to tumor movement. Variant over-actuation of the oncogenic flagging pathway has been embroiled in tumour cell proliferation, endurance, angiogenesis, attack, and metastasis. We in this manner contemplated the job of reserpine in ROS pathways, coming about in over articulation of ROS.

CONCLUSION

In outline, over-actuation of ROS by reserpine can specifically incite apoptosis in NSCLC cells. Moreover, this apoptotic impact is because of enlistment of ROS corruption of cell proliferation, which is intervened by the initiation of cell passing and concealment of oxidative pressure and against apoptotic flagging. In general, our discoveries demonstrated that reserpine is compelling for treating lung cancer.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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