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Lung cancer growth inhibition and autophagy activation by tetrazole *via* ERK1/2 up-regulation and mTOR/p70S6K signaling down-regulation

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Lung cancer, a most common clinically diagnosed malignancy grows rapidly and undergoes metastasis/diffusion to distant organs at a fast rate. In the present study gravacridondiol tetrazole (tetrazole) was synthesized and investigated for lung cancer growth inhibition potential *in vitro*. MTT assay and flow cytometry using propidium iodide were used to determine viability changes and DNA content distribution. Protein expression and apoptotic changes were detected by western blotting and Annexin-V/PI assays. Treatment with 12 μM tetrazole suppressed viabilities to 23% and 20% in A549 and NCI-H1819 cells, respectively. In tetrazole exposed cells, G1-phase cell count increased significantly compared to the control. Tetrazole-treatment of A549 and NCI-H1819 cells caused a prominent raise in LC3-II and p-ERK1/2 expression at 72 h. The SQSTM1/p62 level, p-mTOR and p-p70S6K expression was lowered significantly in A549 and NCI-H1819 cells on exposure to tetrazole. Exposure to U1026 alleviated tetrazole mediated LC3II/I ratio increase in A549 and NCI-H1819 cells significantly (P<0.02) compared to tetrazole treated cells. Treatment with tetrazole and 3-MA in combination led a significant (P<0.02) elevation in A549 and NCI-H1819 cell apoptotic count relative to tetrazole (12 μM) alone treated cells. Moreover, tetrazole and 3-MA combination increased cleavage of caspase-3 to a greater extent compared to tetrazole. In summary, tetrazole manifested anti-proliferative effect on lung cancer cells *via* autophagy over-activation and arrest of cell cycle. It deactivated ERK1/2 signalling and promoted mTOR signaling in A549 and NCI-H1819 cells to regulate cancer proliferation. Thus, tetrazole needs to be studied further as an anti-proliferative agent for treatment of lung cancer.

Keywords: tetrazoles, autophagy, metastasis, anti-proliferative, apoptosis

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Abbreviations: TGF-β, Transforming growth factor-β (TGF-β); DMEM, Dulbecco's modified Eagle's medium; MTT, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; BrdU, bromodeoxyuridine; PDGFRs, Platelet-derived growth factor receptors

INTRODUCTION

Lung cancer, a most common clinically diagnosed malignancy worldwide is detected in two forms: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) (Seymore, 1999). SCLC has been reported to grow rapidly and undergo metastasis/diffusion to distant organs at much faster rate compared to NSCLC (Yang *et al.*, 2009). During the last few decades, several countries including China reported a drastic increase in incidence as well as mortality rate of the lung cancer. Although etiology of lung cancer is not clearly known, reports have found close association of pulmonary cancer with long-term smoking (Bekhit *et al.*, 2004). Among globally diagnosed lung cancer patients ~80% cases account for NSCLC. Clinically it has been demonstrated that there is post-operative recurrence in most of the NSCLC patients undergoing treatment (Bekhit *et al.*, 2004). Patients suffering from advanced stage of NSCLC have very poor prognosis (Shimizu *et al.*, 2004) and therefore effective strategies are needed to control lung cancer development.

Autophagy, a cellular homeostasis process, plays a key role in degradation of dysfunctional/unnecessary contents-proteins or organelles in living beings (Bolt *et al.*, 2012). Autophagy regulates survival response and its over-activation induced by pathogen associated mechanism or due to nutrient starvation becomes fatal for cells as it leads to cell death via a non-apoptotic pathway (Klionsky, 2014). A signalling mechanism identified for autophagy activation in mammalian cells involves mammalian target of rapamycin (mTOR) pathway (Wang *et al.*, 2008). Activation of PI3K/AKT/mTOR signalling has been identified to promote growth, proliferation, and metastasis in different kinds of cancers (Santoni *et al.*, 2013). However, targeting PI3K/AKT/mTOR pathway has been revealed as an appropriate strategy to regulate tumor development (Nazio *et al.*, 2013). Additionally, autophagy activation is also linked to the extracellular signal-regulated kinases 1/2 (ERK1/2) (Hosokawa *et al.*, 2009).

Tetrazole fragment constitutes a promising pharmacophore and is being synthesized in many compounds to improve bioactivity in drug development program (Favata *et al.*, 1998). Flexibility combined with stability on binding to various targets is the main factor which led to advances in tetrazole chemistry (Fukazawa *et al.*, 2002). Another characteristic property that attracts towards tetrazoles as pharmacophores is their ability to act

as carboxylic functionality mimicking agents. Because of this property tetrazoles showed effective anti-inflammatory potential (Sharma *et al*., 2018) and act as analgesics and antiulcer agents (Tang *et al*., 2017). Gravacridondiol is isolated from *Ruta graveolens L*. that is a perennial herb, originally native of the Mediterranean region (Tanida, 2011). Formulations of *R. graveolens* have been used to inhibit progression or to completely repress the glioma growth in human brains without causing toxicity (Klionsky *et al*., 2012). In the present study gravacridondiol tetrazole (tetrazole) was synthesized and investigated for lung cancer growth inhibition potential *in vitro*. Additionally, the mechanism associated with tetrazole mediated cell death was also explored.

MATERIALS AND METHODS

Cell culture

Beas-2B normal epithelial lung cells, A549 and NCI-H1819 lung cancer cell lines were procured from ATCC (Manassas, VA, USA) and grown in DMEM (HyClone, Logan, UT) mixed with FBS (10%). Medium was also mixed with penicillin (100 U/mL) and streptomycin (100 mg/mL). The cells were incubated overnight at 37°C in an incubator under 5% CO_2 and 95% air atmosphere.

Synthesis of gravacridondiol tetrazole (tetrazole)

R. graveolent plant material was obtained from the China-Ladakh boarder and identified by Prof. Zhang Li. Roots of the plant were dried under shade, chopped, and then finely powdered in grinders to collect the powder. The powdered plant material was extracted in ethyl acetate solvent for 48 h, the solvent was decanted and concentrated in rotary vacuum evaporator to obtain the extract. The extract was mixed with silica gel to prepare slurry, which was loaded onto a silica gel column to isolate the gravacridondiol compound.

The solution of gravacridondiol in aqueous alcohol was treated with 1,3-diiodo-5,5-dimethylhydantoin (2 equivalent; DIH) in the dark at 70°C for 12 h. The crude nitrile compound (2; Scheme 1) was purified by column chromatography, dried, and then weighed. The nitrile (2; Scheme 1) obtained was dissolved in DMF and then reacted with sodium azide (2 equivalent) and iodine (6 mol%) for 24 h at 60°C to obtain the desired tetrazole (3; Scheme 1). Impure tetrazole (3; Scheme 1) was loaded onto a column and purified using ethyl acetate/petroleum ether (50:50) solvent system. Tetrazole was characterized using spectral techniques like 1H NMR, HRMS and IR spectroscopy.

Viability assay

The cellular density in 96-well plates was adjusted at 2×10^5 cells/well and grown for overnight in an incubator at 37°C. Tetrazole at 1.5, 3, 6 and 12 μM was mixed with DMEM and cell incubation was performed for 72 h with it. Then, medium from the wells was replaced by fresh medium free from tetrazole. Afterwards, MTT solution (0.5 mg/ml) was poured into each well and incubation of cells was carried out for 4 more hours under the same conditions. Discarding of medium from the wells was followed by an addition of 100 μl DMSO solubilization solution. Gentle shaking of plates to uniformly mix the colour was followed by optical density measurements, in triplicates, at 570 nm using the microplate reader (Wellscan MK3, Labsystems, Finland).

Cell cycle analysis

The cells were distributed at 2×10^4 cells/ well density in 6-well plates and cultured for overnight at 37°C. Cellular distribution in different phases was detected following propidium iodide (Sigma-Aldrich) dyeing of the DNA content. Briefly, trypsinization and then overnight fixing in 70% ethyl alcohol were followed by PBS-washing of the cells two times. Afterwards, centrifugation was performed at $235\times g$ and the separated cells were treated for 15 min with RNase (50 μl) prior to PI (50 μg/ml) staining for 2 h. The FACSAria II instrument for flow cytometry and CellQuest7.6.2 software (BD Biosciences, CA, USA) was employed for the determination of cell fraction in various cell cycle phases.

Western blotting

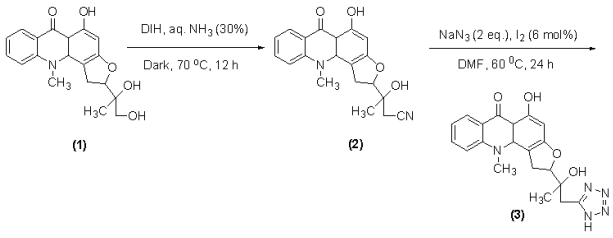
The cells were distributed at 2×10^4 cells/well density in 6-well plates and incubated with tetrazole at 6 and 12 μM for 72 h. Exposure to tetrazole was followed by cell washing two times with cold-PBS and treatment with lysis buffer consisting of NP-40 (1%), sodium phosphate (5 mM), sodium chloride (150), Tris-HCl (pH 7.5; 20 mM), Na_3VO_4 (5 mM), PMSF (1 mM) and leupeptin (10 μg/ml). Lysate formed after 30 min was centrifuged for 20 min at $15\,000\times g$ to collect the supernatants in which protein content was estimated by BCA kits. Samples were loaded in equal amounts (20 μg) to resolve bands on 12% SDS-PAGE followed by transfer onto the PVDF-membranes. Membrane incubation was made at 4°C with primary antibodies overnight, followed by PBS/Tween 20 washing for 1 h. Afterwards, incubation was carried out with the secondary antibodies conjugated to the goat anti-rabbit IgG for 2 h at room temperature. Band visualization was made using SignalFire™ ECL Reagent (Cell Signaling Technology, Inc.) and quantification by Image J version 2.0 software (Bio-Rad Laboratories Inc, USA). Antibodies used were against: p-ERK1/2 (dilution 1:1200), LC3 (dilution 1:1200), sequestosome-1 (SQSTM1/p62; dilution 1:2000), ERK1/2 mTOR (dilution 1:1200), p-mTOR (dilution 1:1200), p-p70S6K (dilution 1:2000) and β-actin (dilution 1:1200; all from Cell Signaling Technology, Inc., Danvers, MA, USA).

Apoptosis assays

Cells were seeded at 2×10^4 cells/well density in 6-well plates and incubated with tetrazole at 12 μM for 72 h. Cells exposed to tetrazole were stained as per instructions of the supplier with Annexin V/PI (BD Biosciences, NJ, USA) for apoptosis detection. Incubation of A549 and NCI-H1819 cells with Annexin-V/PI was performed in the dark for 20 min. Then, apoptosis was observed in cells by a flow cytometer (BD Biosciences, NJ, USA) and data obtained was analysed using WinMDI version 2.5.

Statistical analysis

Data analysis was made using SPSS version-17.0 software (SPSS, Inc., Chicago, IL, USA). All the data obtained in present study are expressed as the mean ± standard deviations of triplicate measurements. One-way analysis of variance (ANOVA) and Tukey's post-hoc test was used for statistical analysis of the data. At $P<0.05$ differences denoted statistically significant differences.



Scheme 1. Synthesis of the gravacridondiol tetrazole.

RESULTS

Tetrazole suppresses A549 and NCI-H1819 cell viability

Tetrazole was synthesised in two steps from gravacridondiol using 1,3-diiodo-5,5-dimethylhydantoin (DIH) as reagent (Scheme 1). Viability changes by tetrazole at 0.75, 1.5, 3, 6 and 12 μM in A549 and NCI-H1819 cells were measured using MTT assay (Fig. 1). In tetrazole-treated cells a significant (P<0.05) suppression in viability was measured in dose-based manner with an increase in the tetrazole concentration. Tetrazole treatment of A549 and NCI-H1819 cells suppressed viabilities to 89% and 86%, respectively at 0.75 μM concentration. Treatment with 12 μM tetrazole suppressed viabilities to 23% and 20%, respectively in A549 and NCI-H1819 cells. Tetrazole treatment did not affect the viability of Beas-2B normal epithelial lung cells (not shown).

Tetrazole leads to A549 and NCI-H1819 cell cycle arrest in G1-phase

Tetrazole exposure at 6 and 12 μM for 72 h was followed by flow cytometric detection of A549 and NCI-H1819 cells for cell cycle changes (Fig. 2A, B). In 12 μM tetrazole exposed A549 cells, G1-phase cell count increased to 71.44±3.98% compared to 43.76±3.14% in control. In NCI-H1819 cells tetrazole exposure at 12 μM raised G1-phase cell population to 75.72±4.52% compared to 43.86±3.61% in the control. Tetrazole exposure caused significant (P<0.05) lowering of cell population in S and G2/M phases in A549 and NCI-H1819 cells at 72 h.

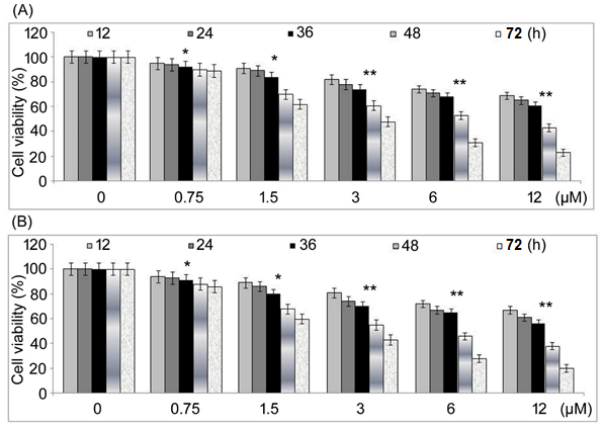


Figure 1. Effect of tetrazole on A549 and NCI-H1819 cell viability.
Exposure to tetrazole at 0.75, 1.5, 3, 6 and 12 μM was followed by determination of viability changes in (**A**) A549 and (**B**) NCI-H1819 cells at 12, 24, 36, 48 and 72 h. *P<0.05, **P<0.02 *vs.* without tetrazole.

Tetrazole promotes LC3-II and lowers SQSTM1/p62 in A549 and NCI-H1819 cells

In tetrazole-treated A549 and NCI-H1819 cells a prominent raise in LC3-II expression was observed at 72 h relative to control (Fig. 3). Tetrazole induced LC3-II expression increase was higher in 12 μM exposed

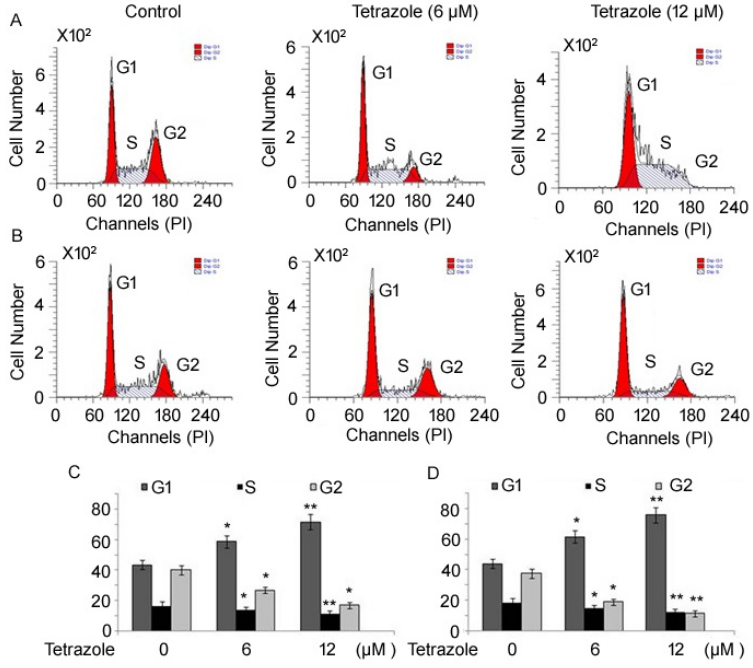


Figure 2. Effect of tetrazole on A549 and NCI-H1819 cell cycle.
Exposure to tetrazole at 6 and 12 μM was followed by flow cytometry for detection of DNA content in (**A, C**) A549 and (**B, D**) NCI-H1819 cells at 72 h. *P<0.05, **P<0.02 *vs.* without tetrazole.

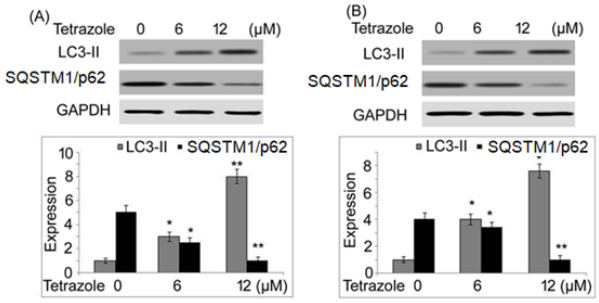


Figure 3. Effect of tetrazole on autophagy markers in A549 and NCI-H1819 cells.
Exposure to tetrazole at 6 and 12 μM was followed by assessment of LC3-II and SQSTM1/p62 expression in (**A**) A549 and (**B**) NCI-H1819 cells at 72 h. *P<0.05, **P<0.02 *vs*. control.

A549 and NCI-H1819 cells compared to cells exposed to 6 μM. The level of SQSTM1/p62 expression showed a marked lowering in A549 and NCI-H1819 cells on exposure to tetrazole. Lowering of SQSTM1/p62 expression by tetrazole treatment was more prominent in 12 μM treated A549 and NCI-H1819 cells relative to 6 μM exposed cells.

Tetrazole regulates ERK1/2 and mTOR phosphorylation in A549 and NCI-H1819 cells

The p-ERK1/2 level showed a prominent enhancement in A549 and NCI-H1819 cells on exposure to tetrazole at 6 and 12 μM (Fig. 4A). In 12 μM tetrazole treated cells, p-ERK1/2 expression was enhanced to maximum level compared to 6 μM treated A549 and NCI-H1819 cells. Exposure of A549 and NCI-H1819 cells to 6 and 12 μM tetrazole significantly lowered p-mTOR expression at 72 h relative to the control (Fig. 4B). Treatment with tetrazole caused significant (P<0.02) inhibition of p-p70S6K expression in A549 and NCI-H1819 cells at 6 and 12 μM doses.

Tetrazole mediated LC3II/I increase in A549 and NCI-H1819 cells is reversed by U0126

Tetrazole treatment at 12 μM significantly (P<0.05) raised LC3II/I ratio in A549 and NCI-H1819 cells relative to control (Fig. 5). However, exposure to U1026 alleviated tetrazole mediated LC3II/I ratio increase in A549 and NCI-H1819 cells significantly (P<0.02) compared to tetrazole treated cells.

Tetrazole promotes A549 and NCI-H1819 cell apoptosis

Significant (P<0.05) rise in apoptotic cell count was observed in A549 and NCI-H1819 cells on treatment with tetrazole at 12 μM (Fig. 6). Exposure to 3-MA could not increase A549 and NCI-H1819 cell apoptotic percentage when compared to the control cells. Treatment with tetrazole (12 μM) and 3-MA in combination led a significant (P<0.02) elevation in A549 and NCI-H1819 cell apoptotic count relative to tetrazole (12 μM) alone treated cells.

Tetrazole mediated A549 and NCI-H1819 cell apoptosis is elevated by 3-MA

In A549 and NCI-H1819 cells tetrazole treatment promoted caspase-3 cleavage markedly at 12 μM doses relative to control cells (Fig. 7). Exposure to 3-MA could not increase caspase-3 cleavage in A549 and NCI-H1819

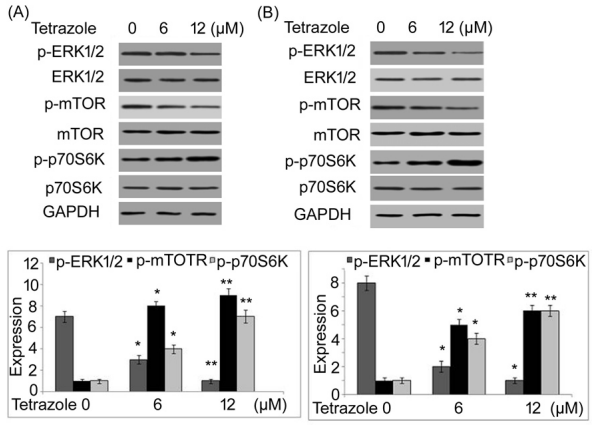


Figure 4. Effect of tetrazole on mTOR/ERK signalling in A549 and NCI-H1819 cells.
Exposure to tetrazole at 6 and 12 μM was followed by assessment of mTOR, ERK and p70S6K activation in (**A**) A549 and (**B**) NCI-H1819 cells. *P<0.05, **P<0.02 *vs*. without tetrazole.

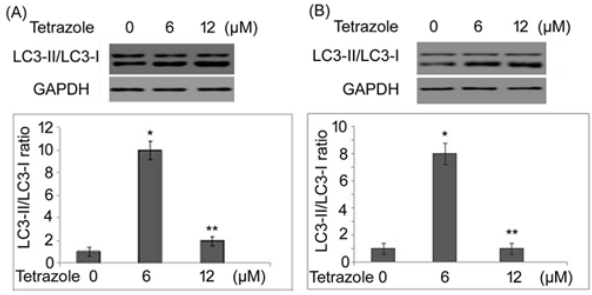


Figure 5. Effect of U1026 on tetrazole induced higher LC3II/I ratio.
A549 and NCI-H1819 cells were treated with tetrazole at 12 μM and then exposed to U1026 at 10 μM doses for 72 h. The LC3II and LC3I expression changes in (**A**) A549 and (**B**) NCI-H1819 cells were monitored by western blotting. *P<0.05, **P<0.02 *vs*. without tetrazole.

cells. However, when A549 and NCI-H1819 cells were treated with tetrazole and 3-MA combination cleaved caspase-3 level showed marked up-regulation relative to tetrazole-treated cells.

DISCUSSION

Chemotherapeutic strategy for cancer has advanced into a novel era wherein molecular pathways are targeted using highly selective drugs that are free from conventional cytotoxicity (Bursch, 2001). Invented tetrazole derivatives were found to be highly active and yielded promising results as anti-cancer agents (Mihaylova *et al*., 2011). Compounds containing tetrazole rings have been demonstrated to exhibit diversity of biological activities (Pyo *et al*., 2012). The present study found that tetrazole repressed A549 and NCI-H1819 cancer cells viability depending upon the concentration added. Tetrazole exposed A549 and NCI-H1819 cells were examined by flow cytometry for possible changes in cell cycle progression. It was found that tetrazole exposure induced an arrest of cell cycle in A549 and NCI-H1819 cells in G1-phase. Tetrazole (12 μM) exposure raised A549 and NCI-H1819 cell population from 43% to more than 70% in the G1-phase. Consequently, significant (P<0.05) lowering of cell population in S and

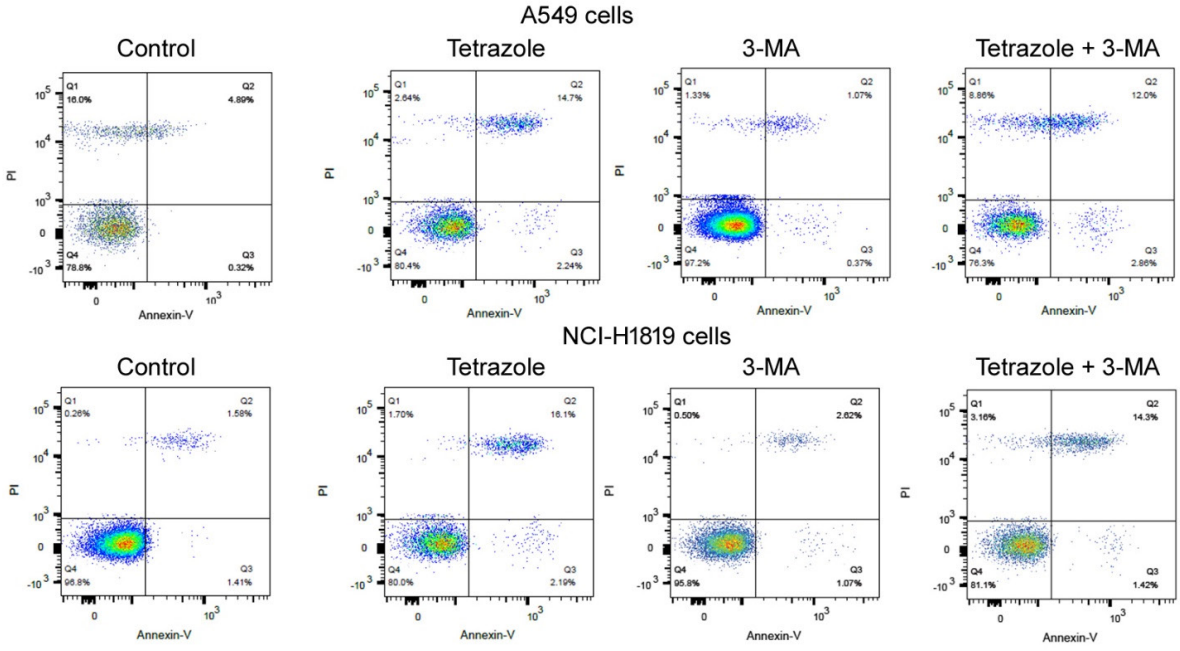


Figure 6. Effect of 3-MA and tetrazole on apoptosis.
A549 and NCI-H1819 cells were treated with tetrazole at 12 µM and then exposed to 3-MA at 5 µM doses for 72 h. Apoptotic death in A549 and NCI-H1819 cells was detected by Flow cytometry. **P*<0.05, ***P*<0.02 *vs.* without tetrazole.

G2/M phases was found in tetrazole exposed A549 and NCI-H1819 cells.

Autophagy is actually a process of homeostasis associated with delivery of various intracellular constituents to be degraded into the lysosomes. Besides several environmental stresses, chemotherapeutics also activate autophagy in tumor cells in different organs (Schmidt-Kittler *et al.*, 2010). Over-activated autophagy acts as another process to eliminate cells in programmed but nonapoptotic way (Kharas *et al.*, 2010). A prominent marker indicating autophagy activation is the expression of LC-3 formed from LC3-I (Dancey *et al.*, 2012). There is also a reduction in p62 level because of its degradation along with other autophagosomal contents during autophagy activation (Cagnol *et al.*, 2010). In the present study tetrazole-treatment of A549 and NCI-H1819 cells led to a prominent raise in LC3-II expression relative to control cells. Moreover, tetrazole mediated LC3-II expression increase was higher in 12 µM exposed A549 and NCI-H1819 cells compared to the cells exposed to 6 µM. Additionally, SQSTM1/p62 level showed a remarkable lowering in A549 and NCI-H1819 cells on exposure to tetrazole. This indicates that tetrazole treatment over-activates autophagy in A549 and NCI-H1819 cells, leading to growth inhibition.

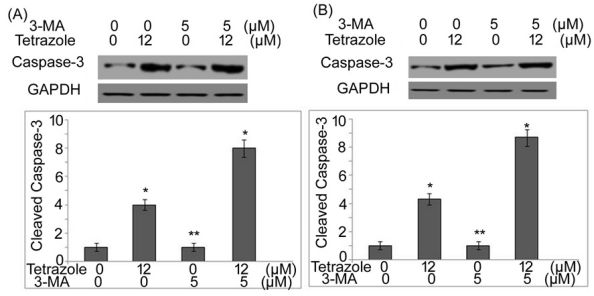


Figure 7. Effect of 3-MA and tetrazole on caspase-3 activation. A549 and NCI-H1819 cells were treated with tetrazole at 12 µM and then exposed to 3-MA at 5 µM doses for 72 h.
Activated caspase-3 was examined in (**A**) A549 and (**B**) NCI-H1819 cells by western blotting. **P*<0.05, ***P*<0.02 *vs.* without tetrazole.

Autophagy induction associated with nutrient starvation or other stress factors is regulated mainly by two pathways such as mTOR and ERK1/2 (Popova *et al.*, 2017). Elevation in p-ERK expression in bladder (J82 & 5637 cells) and adrenocortical carcinoma cells has been demonstrated to over-activate autophagy and inhibit tumor growth (Bachar *et al.*, 2004). It is reported that mTORC1 avoids autophagy *via* deactivation of proteins and induction of mTOR and p70S6Kinase-1 phosphorylation (Natrajan *et al.*, 2010). In the present study p-ERK1/2 level showed a prominent enhancement in A549 and NCI-H1819 cells on exposure to tetrazole at 6 and 12 µM. Exposure of A549 and NCI-H1819 cells to 6 and 12 µM tetrazole significantly lowered p-mTOR expression at 72 h relative to the control. Treatment with tetrazole caused significant ($P<0.02$) inhibition of p-p70S6K expression in A549 and NCI-H1819 cells at 6 and 12 µM doses.

Inhibition of MEK1/MEK2 belonging to MAPK/ERK kinases is achieved in a highly selective manner using U0126 (Hallinan *et al.*, 2002). It is established that U0126 effectively blocks ERK pathway (Meepagala *et al.*, 2005) and because of this A549 and NCI-H1819 cells were exposed to U0126 to confirm tetrazole mediated ERK1/2 activation. In the present study tetrazole treatment significantly ($P<0.05$) raised LC3II/I ratio in A549 and NCI-H1819 cells relative to control. However, exposure to U1026 alleviated tetrazole mediated LC3II/I ratio increase in A549 and NCI-H1819 cells significantly ($P<0.02$) compared to tetrazole treated cells.

Autophagy mediated cell death has been confirmed previously in carcinoma cells during antineoplastic studies (Pathak *et al.*, 2003). The present study used 3-MA to block tetrazole mediated autophagy induction and investigate any increase in cell apoptosis. A significant

($P<0.05$) rise in apoptotic cell count in A549 and NCI-H1819 cells was observed on treatment with tetrazole. Exposure to 3-MA could not increase A549 and NCI-H1819 cell apoptotic percentage when compared to the control cells. Treatment with a combination of tetrazole (12 μM) and 3-MA led to a significant ($P<0.02$) elevation in A549 and NCI-H1819 cell apoptotic count relative to tetrazole alone treated cells. These finding indicated that tetrazole inhibited A549 and NCI-H1819 cell growth via autophagy activation. In A549 and NCI-H1819 cells tetrazole treatment promoted caspase-3 cleavage markedly at 12 μM doses relative to control cells. Exposure to 3-MA could not increase caspase-3 cleavage in A549 and NCI-H1819 cells. However, when A549 and NCI-H1819 cells were treated with tetrazole and 3-MA combination, cleaved caspase-3 level showed marked up-regulation relative to tetrazole-treated cells.

CONCLUSION

In summary, tetrazole manifested anti-proliferative effect on lung cancer cells *via* autophagy over-activation and the arrest of cell cycle. It deactivated ERK1/2 signalling and promoted mTOR signaling in A549 and NCI-H1819 cells to regulate cancer proliferation. Thus, tetrazole needs to be studied further as an anti-proliferative agent for the treatment of lung cancer.

Acknowledgements

Not applicable.

Competing interests

The authors declare that there is no conflict of interest to disclose.

Statement of Ethics

The approval for present study was obtained from the Research Ethics Committee, The Third People's Hospital of Chengdu, Sichuan, China. All the experimental procedures were conducted in accordance with the guidelines issued by National Institute of Health (NIH), USA.

SUMMARY

Treatment with 12 μM tetrazole suppressed viabilities to 23% and 20% in A549 and NCI-H1819 cells, respectively. In tetrazole exposed cells, G1-phase cell count increased significantly compared to the control. Tetrazole-treatment of A549 and NCI-H1819 cells caused a prominent raise in LC3-II and p-ERK1/2 expression. The SQSTM1/p62 level, p-mTOR and p-p70S6K expression was significantly lowered in A549 and NCI-H1819 cells on exposure to tetrazole.

Exposure to U1026 alleviated tetrazole mediated LC3II/I ratio increase in A549 and NCI-H1819 cells significantly ($P<0.02$) compared to tetrazole treated cells. Treatment with tetrazole and 3-MA in combination led a significant ($P<0.02$) elevation in A549 and NCI-H1819 cell apoptotic count relative to tetrazole (12 μM) alone treated cells. Tetrazole and 3-MA combination increased cleavage of caspase-3 to a greater extent compared to tetrazole.

Authors' contributions

Hongyan Zhang conceived and designed the study, Liangjian Zheng, Jun Zhang, Jia Fan, Yuxin He, Tingting Zhan performed experimental work and carried our literature survey. Liwen Rong, Liangjian Zheng and Jun Zhang compiled and analysed the data. Hongyan Zhang and Liangjian Zheng wrote the paper. All the authors approved the paper for publication.

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