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Original article

Valsartan prevents gefitinib-induced lung inflammation, oxidative stress, and alteration of plasma metabolites in rats

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ABSTRACT

Gefitinib (GEF) is an inhibitor of the epidermal growth factor receptor, linked to higher risk of severe/fatal interstitial lung disease (ILD). This study was performed to determine the protective roles of an angiotensin-II type-1 receptor (AT1R) "valsartan (VAL)" in prevention of lung inflammation, oxidative stress and metabolites alteration induced by GEF. Four groups of male Wistar albino rats were received vehicle, VAL (30 mg/kg), GEF (30 mg/kg), or both for four weeks. Blood samples and lungs were harvested for plasma metabolites and histological analysis, respectively, and evaluation of inflammation and oxidative stress. GEF monotherapy showed a dense inflammation in lungs, and significantly increased tumor necrosis factor-α (P = 0.0349), interleukin-6 (P < 0.0001), chemokine ligand-3 (P = 0.0420), and interleukin-1β (P = 0.0377). GEF increased oxidative stress markers including glutathione, malondialdehyde, and catalase levels. Also, several plasma metabolites including butanoic acid, *N*-methylphenylethanolamine, oxalic acid, L-alanine, phosphoric acid, L-theorinine, pyroglutamic acid, and 2-bromosebacic acid were changed by GEF. The combination of VAL plus GEF reduced the inflammation and oxidative stress mediated by GEF monotherapy. In addition, the combination treatment returned plasma metabolites to the normal levels compared to GEF monotherapy. These findings revealed that VAL has a possible pulmonary protective role against pulmonary toxicity of GEF, which may lead to novel approaches for management of GEF-induced ILD.

1. Introduction

The second most prevalent cancer diagnosis is lung cancer (Thandra et al., 2021). Globally, lung malignancy leads to death among males and females, with an estimated 2.1 million new cases and 1.8 million deaths each year (Sung et al., 2021). The histological subtype, non-small cell lung cancer carcinoma (NSCLC), comprises around 85 % of all diagnosed patients (Molina et al., 2008; Mirhadi et al., 2021). In addition to surgery, combination therapy of systematic cytotoxic agents with platinum-based treatment remains the cornerstone therapy for management of late-stage NSCLC (Hanna et al., 2017). Limited long-term survival and dose-limiting toxicities have urged to development of more effective and less toxic systemic treatments, especially in patients with driver genetic mutations in EGFR, an activating mutation that can be found in about one-third of NSCLC cases (Zhang et al., 2016). The transmembrane receptor tyrosine kinase, EGFR, is a primary oncogenic driver of several downstream cancer hallmarks (Sigismund et al., 2018). Tyrosine kinase inhibitors (TKIs) provide significant clinical benefits in cases harboring EGFR mutation (Solassol et al., 2019).

Various degrees of adverse effects were noticed in patients using TKIs, ranging from dermatological reactions and diarrhea to more serious ones such as ocular toxicity and hepatic dysfunction (Takeda et al., 2012; Ding et al., 2017; Shah et al., 2019). Although it is less common, the incidence of life-threatening pulmonary toxicity, ILD in particular, has been increasingly reported as a result of the TKIs treatment. Qi, W. X et. al reported that all-grade ILD has

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been associated to EGFR-TKIs (1.6 %; 95 % CI, 1.0–2.4 %), high-grade ILD (0.9 %; 95 % CI, 0.6 %-1.4 %), and mortality (13.0 %; 95 % CI, 7.6–21.6 %) (Qi et al., 2015). Mounting evidence has also reported a broad spectrum of GEF-mediated adverse pulmonary toxicities, including pneumonia and, more seriously ILD (Inoue et al., 2003; Hotta et al., 2005; Akamatsu et al., 2013; Beom et al., 2016). A *meta*-analysis study of 17 clinical trials revealed that receiving GEF treatment was significantly related with an increased incidence of ILD (1.5 % of 8609 patients) (Ohmori et al., 2021). In addition, analyzing a total of 26 clinical trials revealed that the mortality rate of ILD-related deaths with either GEF or erlotinib treatments for advanced NSCLC was 22.8 % compared to controls at 7.1 % (Shi et al 2014). Results from another study revealed that GEF was linked to a considerably higher incidence of ILD 1.43 % (95 % CI: 0.98 %–2.09 %) compared with the controls (Hong et al., 2016).

The etiology of this form of drug-induced pulmonary injury has been linked to several pathways, including oxidative stress leading to dysregulated redox status, and cytokines production (Higenbottam et al., 2004; Matsuno et al., 2012). The correlation between inflammation and GEF-induced ILD was insinuated by the observation that patients treated with high-dose corticosteroids showed a faster improvement (Kataoka et al., 2006; Seto et al., 2006; Hong et al., 2016).

Although known as a main controller of hemodynamic function and nutritional homeostasis (Ferrario et al., 2006; Aimo et al., 2021), the renin-angiotensin-aldosterone system (RAS) is increasingly implicated in induction of inflammation leading to the injury/repair response at the tissue level (Marshall et al., 2003; Aimo et al., 2021). Several studies have associated alterations in local RAS expression and activity with different lung-related diseases, including idiopathic pulmonary fibrosis (Montes et al., 2012), pulmonary sarcoidosis (Song et al., 2015), pulmonary hypertension (de Man et al., 2012), and lung cancer (Rosenthal et al., 2019). Angiotensin II (AngII), as a bioactive RAS product, has been linked to the initiation and maintenance of lung disorders (Shenoy et al., 2010). In the lung interstitium, the elevated levels of angiotensin-converting enzyme (ACE) of the diseases mentioned above support the notion of AngII's function in pulmonary diseases (Marshall et al., 2003). Miao L. et al. have studied the influence of ACE inhibitors (ACEIs) and AT1R blockers (ARBs) in NSCLC subjects treated with TKIs (Miao et al., 2016). Despite the lung cancer stage, they found a notable improvement in the survival rate of subjects that received ACEI/ARB compared to non-treated group, even in subjects who received TKIs (Miao et al., 2016). More recently, findings from an in vivo study showed that using VAL prevented lung inflammation and fibrotic scarring induced by bleomycin (Mojiri-Forushani et al., 2018). We, therefore, conducted the present study to identify the impact of using VAL in GEF-mediated pulmonary toxicity in rat animal model. We hypothesized that VAL would prevent inflammation, oxidative stress, metabolites disturbances and subsequent lung injury by inhibiting the release of inflammatory mediators and cytokines.

2. Materials and methods:

2.1. Animal

Wistar albino rats (male, 200 ± 20 g) were provided from the Animal Care Center, College of Pharmacy, King Saud University (Riyadh, KSA), and had free access to water and a normal diet under a regular12/12hr of light/dark cycle. All procedures were approved by the local institutional research ethics committee of King Saud University (KSU-SE-21–22), in accordance with ARRIVE guidelines.

2.2. Treatment

Four groups of thirty-two rats (n = 8 in each group) were treated for continuous 4 weeks. First group was treated with vehicle (1 % DMSO, *o.p.* and saline/0.1 M NaOH, *i.p.*) and considered as a control group. The second group was received VAL (30 mg/kg/day, *i.p.*) that dissolved in saline/0.1 M NaOH (Jiao et al., 2012). The third group was treated with vehicle for a week and then received GEF (30 mg/kg/day, *o.p.*) for three weeks. The last group was received VAL for a week and then received GEF and VAL for three weeks. The dose of VAL and GEF were chosen based on previous research papers (Alhazzani et al., 2021; Alanazi et al., 2022). All animals were weighed daily for calculation of medication doses and weight gain percentage in all groups. After one month of treatment, all groups were injected via with ketamine/xylazine mixture to induce anesthesia. Then, blood samples were withdrawn directly from the heart to collect plasma for biochemical analysis. Lungs were harvested and weighed to measure the lung/body weight ratio as an index of lung damage (Ruan et al., 2020). Then, the collected lungs were immersed into formaldehyde for histopathology study, and the remaining lungs were homogenized and stored at −80 °C for further studies.

2.3. Lung histology

At the time of euthanasia, lungs from all groups were collected and immediately preserved in a 4 % formaldehyde solution. Lungs were fixed in a paraffin block and cut at 3 μm using microtome. The collected slices were immersed in xylene and alcohol for deparaffinization and hydration before staining via hematoxylin and eosin (H/E). A professional histopathologist examined the produced slides under an optical microscope (Olympus BX microscope and DP72 camera) to discover structural alterations in the lung tissues.

2.4. Real-time quantitative polymerase chain reaction (RT-qPCR) analysis

The collected lungs were homogenized into TRIzol (Invitrogen, CA, USA) to extract the cellular RNA from the pulmonary tissues. RNA purity and concentration were assessed by a NanoDrop™ 8000 spectrophotometer (Thermo Scientific, USA). Strands of complementary deoxyribonucleic acid (cDNA) were synthesized from the total RNA by a TaqMan™ Reverse Transcription kit (ThermoFisher Scientific, USA). RT-PCR was conducted utilizing a SYBR Green PCR master mix (Bimake, USA) and QuantStudio 6 Flex real-time PCR System (ThermoFisher Scientific, USA), and the fold change was determined using the $2^{-\Delta\Delta CT}$ method. Rat interleukin-6 (IL-6), chemokine ligand-3 (CCL3), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) primers were purchased from Integrated DNA Technologies (Coralville, IA). The following primers were used: IL-6 forward 5′- GCC CTT CAG GAA CAG CTA TGA −3′, IL-6 reverse 5′- TGT CAA CAA CAT CAG TCC CAA GA −3′, CCL2 forward 5′- AAG AAG CTG TAG TAT TTG TCA CCA AGC TCA −3′, CCL2 reverse 5′- CAT CAG GTA CGA TCC AGG CT −3′, IL-1β forward 5′- CCA GAT GGA GTT TGA GGA CCC AAG CA −3′ IL-1β reverse 5′- TCC CGA CCA TTG CTG TTT CC −3′, TNF-α forward 5′- CAC GCT CTT CTG TCT ACT GA −3′ TNF-α reverse 5′- CTA CCA CCA GTT GGT TGT CT −3′, β-actin forward 5′- CCA GAT CAT GTT TGA GAC CTT CAA −3′, β-actin reverse 5′- GTG GTA CGA CCA GAG GCA TAC A −3′.

2.5. Western blot analysis

The pulmonary samples were lysed into radioimmunoprecipitation assay (RIPA) buffer (ThermoFisher Scientific, USA). The homogenate of each sample was loaded into gels (10 % SDS-PAGE gels)

for proteins separation, and then using nitrocellulose membranes for transferring the separated proteins. Membranes were immersed into a blocking solution containing Tris-buffer saline, 0.1 % Tween-20, and 2 % bovine albumin serum (pH 7.4), and kept for 2 hrs (Alanazi et al., 2021). After that, overnight incubation of the membranes with primary antibodies was performed at 4 °C for the targeted proteins; IL-6, CCL3, IL-1β, TNF-α, or GAPDH (ABclonal Technology, USA) followed by 1 hr incubation with secondary antibodies at room temperature. Membranes were subjected to chemiluminescent HRP substrate for bands detection.

2.6. Measurement of oxidative stress biomarkers in lung tissues

The supernatant of each pulmonary sample was collected after tissue homogenization in phosphate buffer, and then aliquoted for determination of glutathione (GSH), malondialdehyde (MDA), and catalase (CAT) concentrations in pulmonary tissues of all treated groups following previous protocols (Imam et al., 2018).

2.7. Gas chromatography-mass spectroscopy (GC–MS)

Plasma samples will be analyzed by GC–MS to study the effect of GEF with and without VAL treatment on plasma metabolites. As previously described, a plasma sample of each group (100 µL) was mixed with 250 µL of acetonitrile and centrifuged (10,000 rpm) to participate the protein. The supernatant (400 µL) was evaporated using nitrogen gas, then adding methoxylamine hydrochloride (20 % v/v in pyridine) to the vial and kept for 1 hr at 60 °C, then mixed with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 1 % trimethylchlorosilane (TMCS) for 1 hr at 70 °C. The final

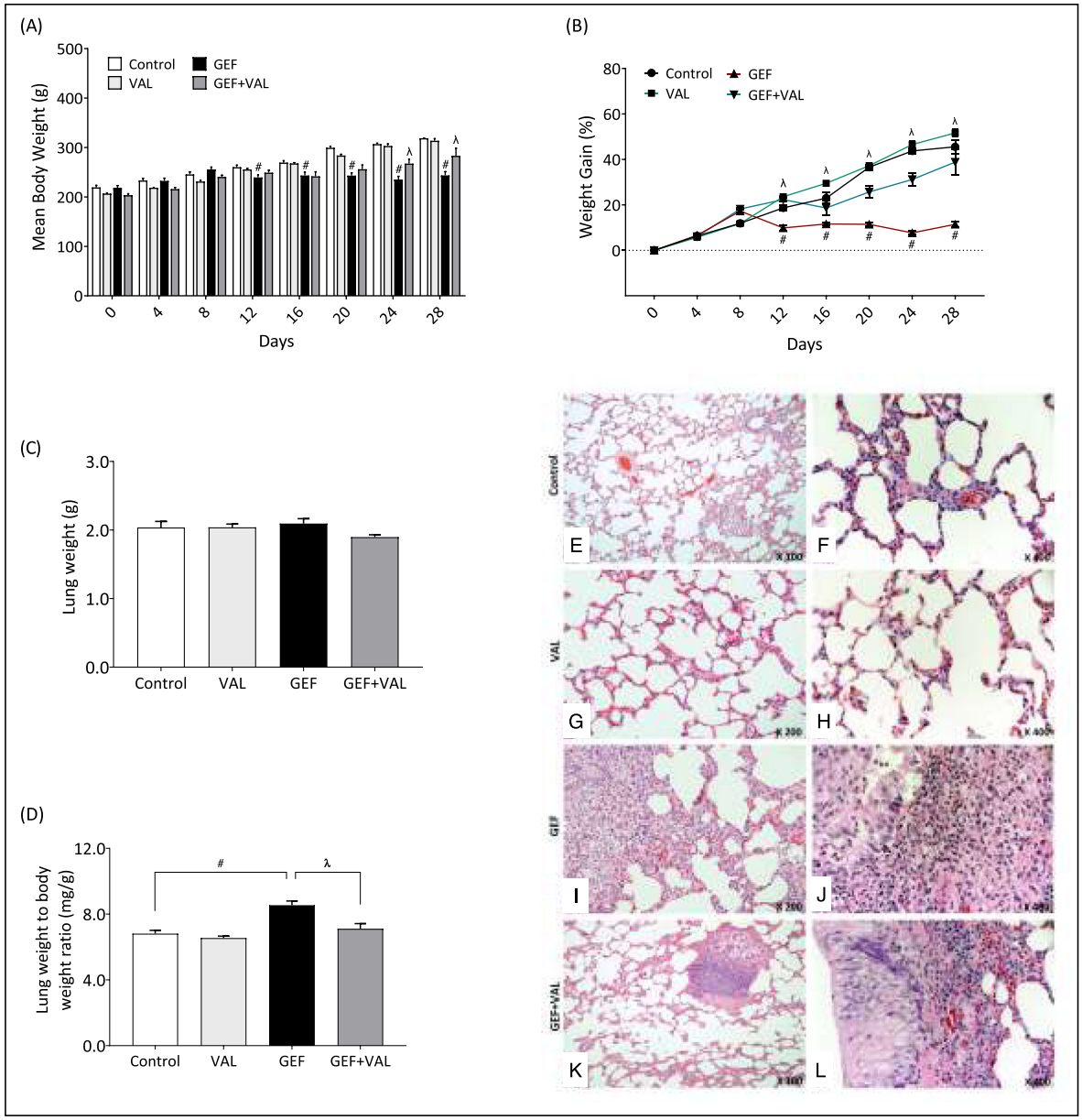

Fig. 1. Effect of gefitinib and/or valsartan on body/lung weight and lung histology. Rats groups were treated with vehicle (control), VAL (30 mg/kg, i.p.), GEF (30 mg/kg, o.p.), or GEF + VAL. Body weight (Panel A) and weight gain percentage (Panel B) were measured every 4 days. Lung weight (Panel C) and Lung/body weight ratio (Panel D) were measured after lungs collection. Graph and linear bars indicate mean ± SEM for the following treatment groups (n = 6 rats/ each group): Control (white bars); VAL (light fray fill bars); GEF (black bars); VAL + GEF (dark gray bars). $^{\#}P < 0.05$ for control vs GEF; $^{\lambda}P < 0.05$ for GEF vs GEF + VAL. Lung histology was performed using hematoxylin and eosin staining (H/E) (Panel E-K). Note the presence of dense inflammation in a lung section obtained from the lung of a GEF treated group, including many foamy macrophages on the left side of the photomicrograph (Panel I-J). In the lungs of a combined GEF and VAL treated group, note the marked improvement in the intensity of inflammation (Panel K-L). In the lungs of control and VAL groups, note the preservation of normal lung histology and architecture. H/E stain x100-400. VAL, valsartan; GEF, gefitinib.

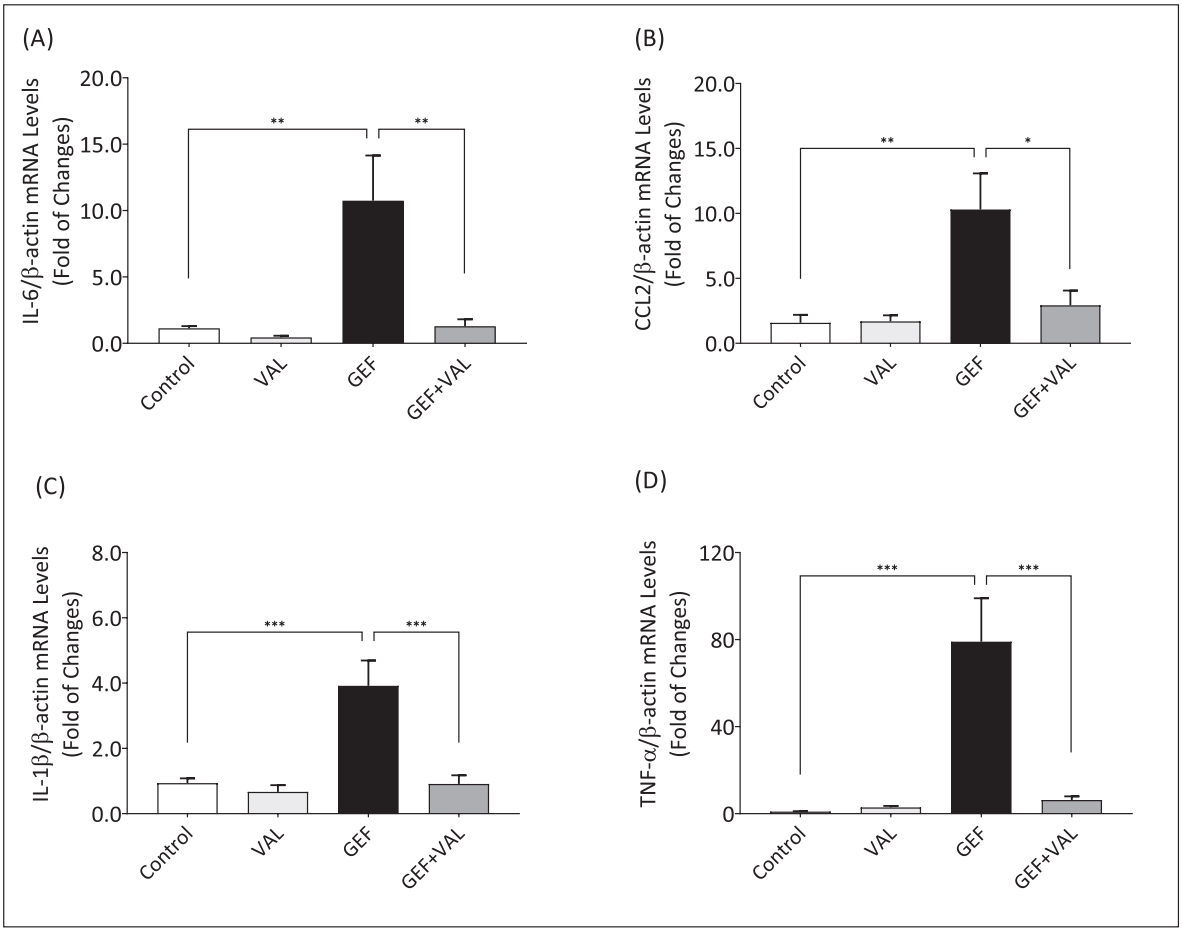

Fig. 2. Effect of gefitinib and/or valsartan on mRNA levels of inflammatory mediators. Rats groups were treated with vehicle (control), VAL (30 mg/kg, i.p.), GEF (30 mg/kg, o.p.), or GEF + VAL. mRNA was extracted from animals' lung tissues. RT-qPCR was used for measuring mRNA levels of the following inflammatory markers: IL-6 (Panel A), CCL2 (Panel B), IL-1β (Panel C), and TNF-α (Panel D). Graph bars indicate mean fold change of mRNA level measures ± SEM for the following treatment groups (n = 5 rats/ each group): Control (white bars); VAL (light fray fill bars); GEF (black bars); VAL + GEF (dark gray bars). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. VAL, valsartan; GEF, gefitinib.

product was mixed with *N*-heptane, and centrifuged for 10 min. The supernatant was collected and analyzed by GC–MS.

2.8. Statistical analysis

The collected data were analyzed by GraphPad Prism (version 9.0). The comparison between all groups was conducted via one and two ANOVA followed by the Tukey-Kramer multiple comparison test. For metabolomics, the heat map was designed by MetaboAnalyst web service (https://www.metaboanalyst.ca). The obtained data were illustrated as mean ± SEM and P value < 0.05 was considered to estimate the significance statements between treatments.

3. Results:

3.1. Effect of gefitinib and/or valsartan on body weight, lung weight and relative parameters and lung histology

Body weight (BW) and weight gain percentage (WG%) were measured every 4 days until the end of the study. GEF decreased both BW and WG% as compared to the control group (Fig. 1A-B). Adding VAL attenuated the reduction in BW and WG% of rats treated with GEF in the GEF + VAL group (Fig. 1A-B). Lung weight and Lung/body weight (LW/BW) ratio were measured in all treated groups (Fig. 1C-D). LW/BW ratio was markedly increased during GEF treatment in comparison with the control. In contrast, cotreatment with VAL notably normalized the LW/BW ratio in the GEF + VAL group (Fig. 1D). All these parameters show no significant changes between control and VAL groups (Fig. 1A-D).

In the histological study, GEF treatment showed a dense inflammation in the lung tissue including many foamy macrophages on the photomicrograph's left side (Fig. 1I-J). Concomitant treatment of VAL with GEF notably decreased the induced inflammation by GEF as compared with the GEF group (Fig. 1K-L). In the control and VAL groups, results showed normal lung histology and architecture (Fig. 1E-H).

3.2. Effect of gefitinib and/or valsartan on mRNA levels of inflammatory mediators

Fig. 2A, B, C, and D show that VAL alone, in the treated group no. 2, did not affect the mRNA level in all inflammatory markers: IL-6, CCL3, IL-1β, and TNF-α. GEF, in contrast, raised the amount of all four inflammatory mediators significantly. However, the increased levels of those markers were normalized notably by adding VAL to GEF in the GEF + VAL group.

3.3. Effect of gefitinib and/or valsartan on protein levels of inflammatory mediators

Protein concentrations of IL-6, CCL3, IL-1β, and TNF-α were assessed using Western blotting technique (Fig. 3A, B, C, and D). Outcomes illustrate that GEF caused a remarkable increase in all

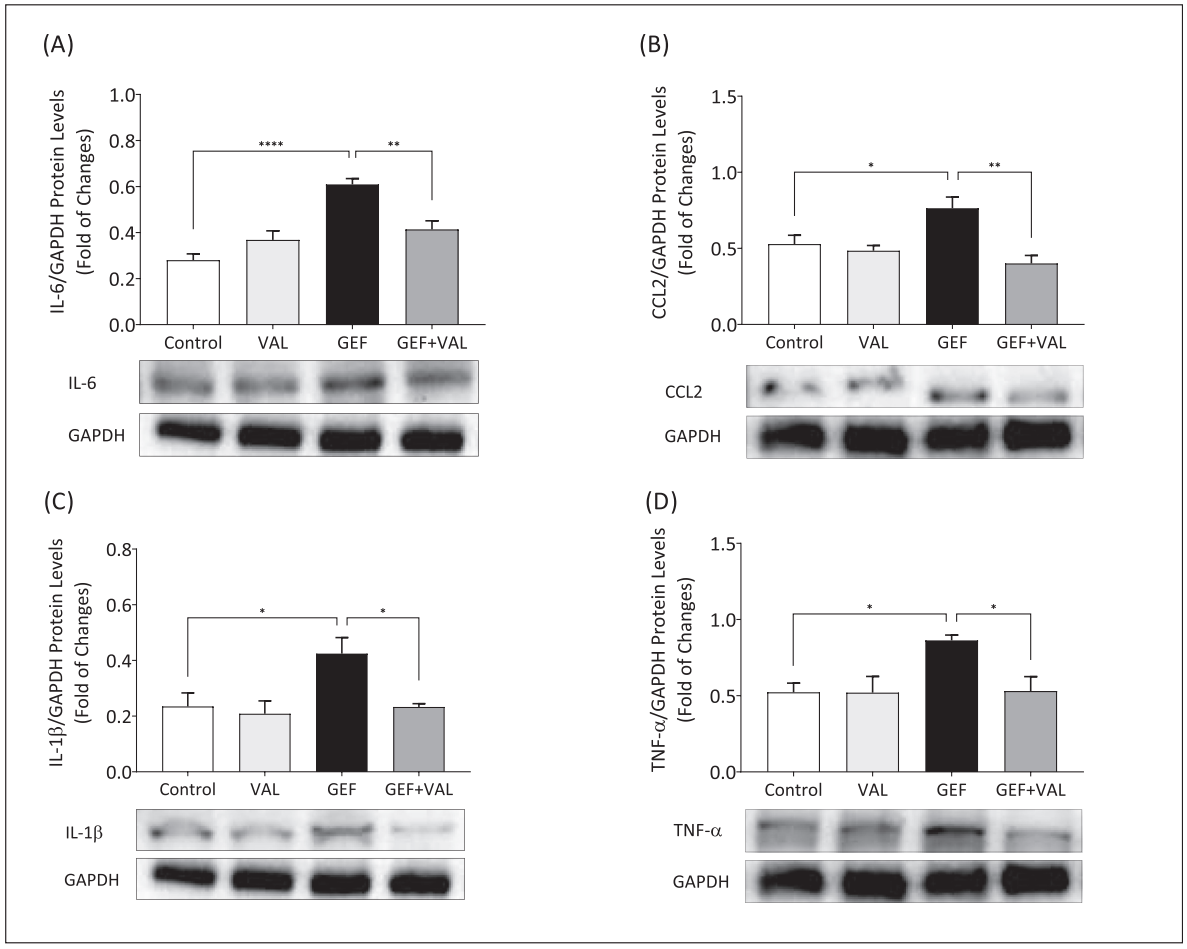

Fig. 3. Effect of gefitinib and/or valsartan on protein levels of inflammatory mediators. Rats groups were treated with Vehicle, VAL (30 mg/kg, i.p.), GEF (30 mg/kg, o.p.), or GEF + VAL. Lung tissues were collected and analyzed using western blot for the following proteins: IL-6 (Panel A), CCL2 (Panel B), IL-1β (Panel C), and TNF-α (Panel D). Bars show mean fold change of protein level measures ± SEM for the following treatment groups (n = 5 rats/ each group): Control (white bars); VAL (light fray fill bars); GEF (black bars); VAL + GEF (dark gray bars). Targeted protein with GAPDH blot is depicted below each graph. *$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$. VAL, valsartan; GEF, gefitinib.

four inflammatory indicators protein measures. Adding VAL to GEF, obviously, had restored the levels of all the abovementioned proteins.

3.4. Effect of gefitinib and/or valsartan on oxidative stress

The levels of GSH, CAT, MDA were considerably altered in lungs after three weeks of GEF administration (Fig. 4A-C). GEF decreased the antioxidants levels (GSH and CAT) while increasing the oxidant levels (MDA) in lungs. In contrast, VAL restored these markers to normal levels and prevented the oxidant/antioxidant imbalance caused by GEF in the GEF + VAL group (Fig. 4A-C).

3.5. Effect of gefitinib and/or valsartan on plasma metabolomics profile

Gas chromatography coupled with mass spectrum was deployed to identify changes in plasma metabolites among various treatment groups. Heat map analysis was generated using Metaboanalyst as a comparative tool to identify changes in metabolites in the control, VAL, GEF, and GEF + VAL treated rats. As shown in Figure 5A, a total of 20 metabolites were found to be disrupted in various treatment groups. Among these metabolites, we found 8 metabolites that have highly discriminating levels including butanoic acid, *N*-methylphenylethanolamine, oxalic acid, L-alanine, phosphoric acid, L-theorinine, pyroglutamic acid, 2-bromosebacic acid (Fig. 5B-I).

4. Discussion:

TKIs have ushered in a new era in cancer management. ILD is one of the most significant of their numerous side effects, occurring most commonly (Shah, 2016). Around 57 % of the current approved TKIs have been implicated in induction of ILD with varied degrees of frequency and/or severity (Shah, 2016). Despite the fact that GEF is a relatively safe oral anti-cancer drug, pulmonary toxicity has been documented as a significant side effect, and the mechanism underlying GEF-induced ILD is yet to be fully understood (Kataoka et al., 2006). The purpose of this experimental research was to examine the function of AT1R blockade via VAL in attenuation of GEF-induced lung toxicity in a rat model. The finding results identified that three weeks of GEF treatment led to a reduction in BW and WG%, which might be correlated to the unhealthy condition of rats caused by GEF treatment. In addition, GEF increased the LW/BW ratio, which is a measurement of cellularity and edema to estimate the extent of lung damage (Ruan et al., 2020). The histological study showed that GEF induced inflammation in lung tissues including many foamy macrophages. On the molecular levels, GEF activated the production of inflamma-

tory mediators including IL-6, CCL3, IL-1β, and TNF-α. Recent studies demonstrated that GEF initiates the activation of inflammation and interstitial pneumonitis via IL-1β production and high-mobility group box1 (HMGB1) in macrophages leading to induction of the NLRP3 inflammasome and mitochondrial oxidative stress, respectively (Kagi et al., 2021; Noguchi et al., 2021).

In contrast, AT1R blockade by VAL in GEF treated rats regulated the associated disturbances in BW, WG% and LW/BW ratio. Our finding revealed that VAL attenuated GEF-induced inflammation, which was approved through histological examination and mRNA and protein analysis of IL-6, CCL3, IL-1β, and TNF-α. Results in the current study were consistent with previous studies showed that VAL reduced inflammatory mediators in various pathological conditions associated with anticancer drugs, blood sepsis, and cardiovascular and pulmonary diseases (Cole et al., 2010; Iwashita et al, 2013; Lu et al., 2017; Mojiri-Forushani et al., 2018). Recent study found that VAL attenuated smooth muscle cell proliferation, extracellular matrix remodeling and inflammatory mediators including MMP-2, MMP-9 and TNF-α in lungs of pulmonary hypertensive animal models (Lu et al., 2017). Previous research showed that VAL supressed IL-6 and TNF-α release in blood sepsis caused by lipopolysaccharide and restored them to the normal levels (Iwashita et al, 2013). In cancer therapy, a study approved that VAL attenuated production of interleukin-4 and interferon-γ, and gene expression of nuclear factor-kappaB (NF-κB) in bleomycin-induced pulmonary fibrosis (Mojiri-Forushani et al., 2018). In addition, studies have shown that VAL has a potential role in downregulation of inflammatory mediators in inflammatory bowel disease and autoimmune myocarditis (Santiago et al., 2008; Liang et al., 2021). Still, the molecular mechanism underlying AT1R blockade prevents GEF-induced lung inflammation remains unclear.

Moreover, GEF disturbed oxidants/antioxidants balance leading to abnormal levels of GSH, CAT and MDA in lung tissues. This finding was consistent with a previous study indicated that GEF decreased glutamine levels, which provides precursors to produce GSH, leading to oxidative stress and cell death in GEF-sensitive PC-9 cells (Wang et al., 2018). It was recently revealed that giving GEF to mice after they had been exposed to naphthalene exacerbated their lung damage (Terasaki et al., 2019). GEF/naphthalene induced production of inflammatory mediators, lipid peroxidation and GSH depletion increased the severity of their pulmonary injury (Terasaki et al., 2019). The exact mechanism of GEF-mediated oxidative stress remains to be elucidated. In contrast, several research papers showed the role of AT1R blockers in providing the balance between ROS, GSH, CAT and lipid peroxidation in cardiovascular diseases (Wu et al., 2013; Cheng et al., 2020). Pretreatment with VAL regulated MDA and superoxide dismutase (SOD) content providing cardioprotection in ischemia (Wu et al., 2013). Adding VAL with doxorubicin treatment attenuated ROS production and oxidative stress showing protective effects against doxorubicin-induced cardiotoxicity (Cheng et al., 2020). In lungs, VAL prevented ROS in cyclophosphamide-induced pulmonary injury through downregulation of MAPK and NF-κB (Abdel-Latif et al., 2020).

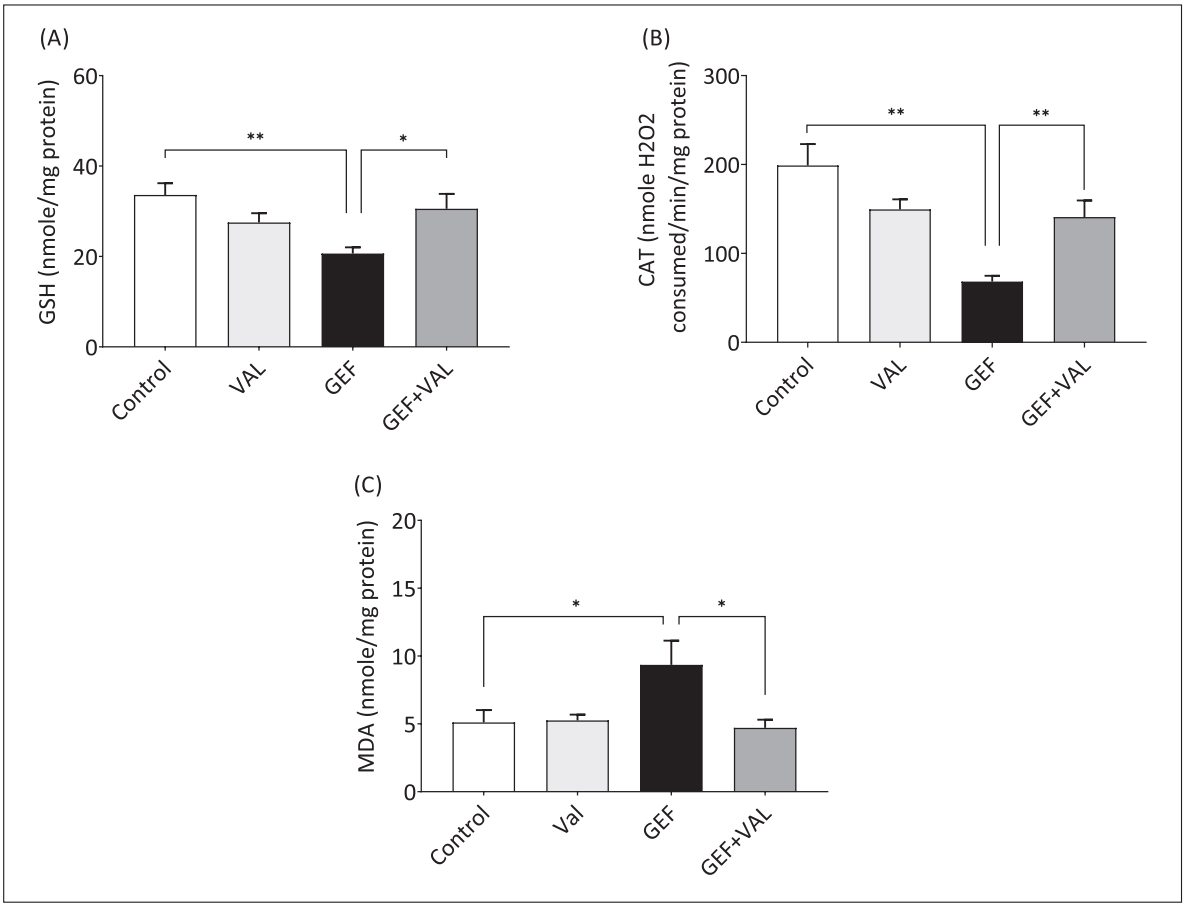

Fig. 4. Effect of gefitinib and/or valsartan on oxidative stress markers. Rats groups were treated with Vehicle, VAL (30 mg/kg, i.p.), GEF (30 mg/kg, o.p.), or GEF + VAL. Lung tissues were collected and analyzed the following oxidative stress markers: GSH (Panel A), CAT (Panel B) and MDA (Panel C). Bars show mean ± SEM for the following treatment groups (n = 6 rats/ each group): Control (white bars); VAL (light fray fill bars); GEF (black bars); VAL + GEF (dark gray bars). $^{*}P < 0.05$; $^{**}P < 0.01$. VAL, valsartan; GEF, gefitinib.

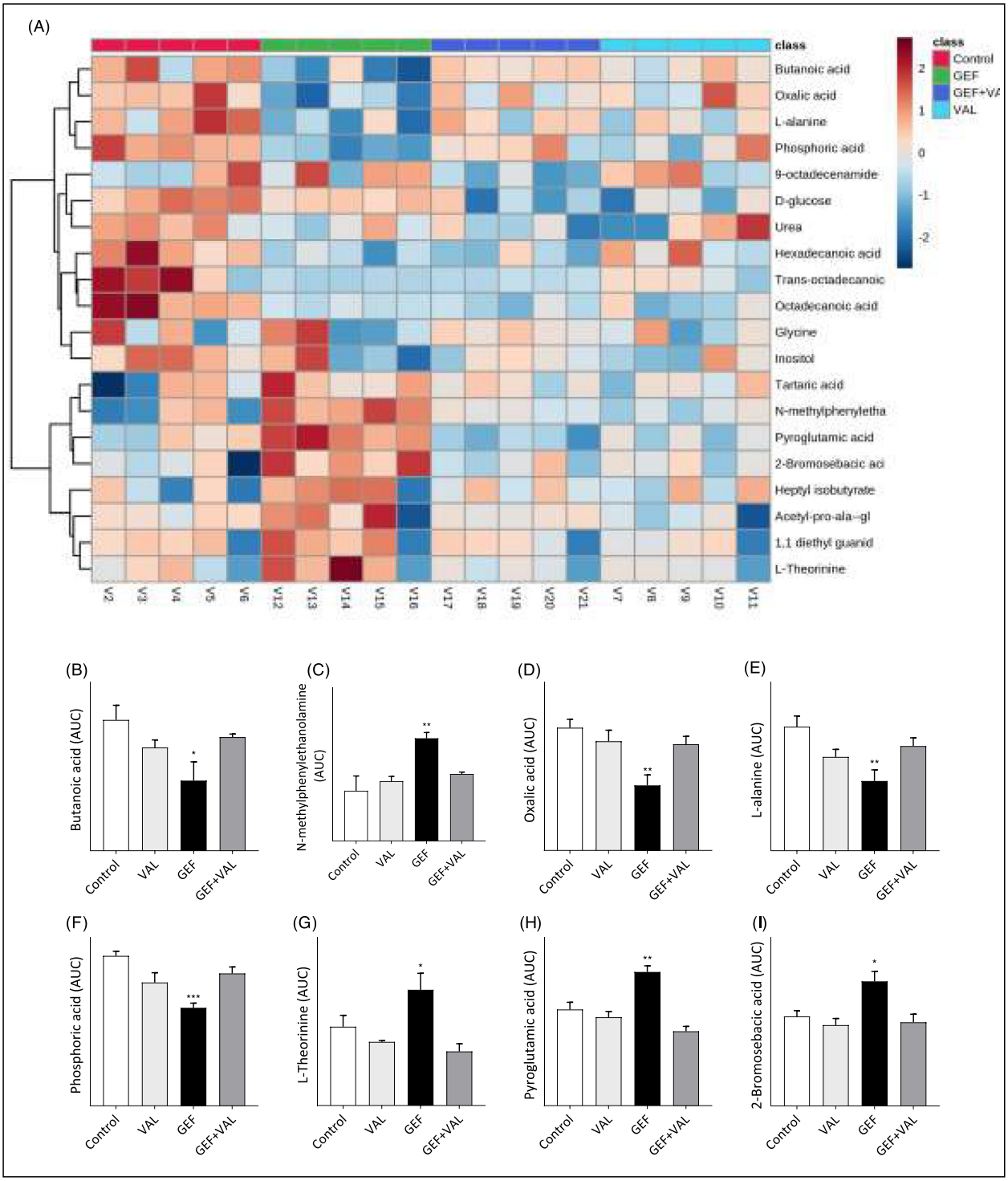

Fig. 5. Plasma metabolomics analysis identifying changes in plasma metabolites associated with gefitinib and/or valsartan. Rats groups were treated with Vehicle, VAL (30 mg/kg, i.p.), GEF (30 mg/kg, o.p.), or GEF + VAL. (Panel A) heat map analysis showing the expression levels of 20 metabolites in various treatment groups. Blue box indicates low expression levels whereas red box indicates high expression. (Panel B-I) bar graphs for selected plasma metabolites associated with control, VAL, GEF, and GEF + VAL treatment. Graph bars indicate mean ± SEM for the following treatment groups (n = 5 rats/ each group): Control (white bars); VAL (light fray fill bars); GEF (black bars); VAL + GEF (dark gray bars). *P < 0.5; **P < 0.01; ***P < 0.01 compared to control.

In the current study, plasma samples were examined using a gas chromography coupled with mass spectrum to identify changes in the metabolic profile of VAL, GEF and the combination of GEF + VAL. The metabolomics analysis provided a clear separation of certain metabolites in GEF treated rats in comparison to the control and VAL groups. Among these metabolites, GEF has significantly decreased the levels of butanonic acid, oxalic acid, L-alanine, phosphoric acid whereas the levels of L-theorinine pyroglutamic acid, and 2-brmosebacic acid have significantly increased as compared to control, VAL, or GEF + VAL. Several reports have linked lung inflammation and colon inflammation with decrease in energy derived metabolites such as butanoic acid and oxalic acid

(Kalina et al., 2000; Hamer et al., 2008; Singh et al., 2014; Vieira et al., 2019). Butanoic acid is a short chain fatty acid that is mainly metabolized by mitochondria through fatty acid oxidation in order to produce energy. It has been found that treatment with butanonic acid would attenuate pulmonary inflammation and mucus formation in animals treated with ovalbumin (Vieira et al., 2019). Similarly, oxalic acid inhibits glycolysis, a process of oxidation of a glucose molecule to yield a pyruvate molecule which can later be utilized in kreb's cycle to produce more energy.

(Buc et al., 1981). Hypophosphatemia is also associated with inflammatory lung illnesses including chronic obstructive diseases (Stroda et al., 2018). Thus, our results indicate that GEF may induce lung inflammation by disruption of energy related metabolites as well as lower phosphoric acid. In addition, pyroglutamic acid is an intermediate substrate involved in the synthesis of GSH. It has been shown that glutathione depletion caused by acetaminophen induced toxicity can lead to pyroglutamic acid accumulation. Similarly, GEF treatment has significantly elevated plasma pyroglutamic acid levels suggesting that GEF might deplete GSH levels (Emmett, 2014).

5. Conclusion

Our findings suggest that VAL attenuates pulmonary inflammation induced by GEF through suppressing inflammatory mediators. In addition, VAL decreased the oxidative stress induced by GEF via regulation GSH, MDA and CAT levels in lungs. Analysis of plasma metabolites showed that VAL regulated altered metabolites associated with inflammation and oxidative stress during GEF treatment such as butanoic acid and pyroglutamic acid, respectively. Thus, the current study showed the potential roles of AT1R blockade via VAL to reverse the pulmonary toxicity of GEF. Still, further studies are required to clarify the role of GEF and VAL on plasma metabolites and the mechanism underlying GEF-induced RAS leading to pulmonary toxicity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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