



Preliminary evaluation of anticancer efficacy of pioglitazone combined with celecoxib for the treatment of non-small cell lung cancer

Ammu V. V. V. Ravi Kiran¹ · Garikapati Kusuma Kumari¹ · Praveen T. Krishnamurthy¹ 

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Summary

Purpose. Among the lung cancer types, non-small cell lung cancer (NSCLC) is prominent and less responsive to chemotherapy. The current chemotherapeutics for NSCLC are associated with several dose-limiting side effects like bone-marrow suppression, neurotoxicity, nephrotoxicity, and ototoxicity, etc. which are causing non-compliance in patients. Many tumors, including breasts, lung, ovarian, etc. overexpress PPAR- γ receptors and COX-2 enzymes, which play a crucial role in tumor progression, angiogenesis, and metastasis. Lack of PPAR- γ activation and overproduction of prostaglandins, result in uncontrolled activation of Ras/Raf/Mek ultimately, NF- κ B mediated tumor proliferation. This study aimed to investigate the anti-cancer potential of PPAR- γ agonist Pioglitazone combined with COX-2 inhibitor Celecoxib in NSCLC. **Methods.** Sixty adult Balb/C male mice were classified into sham control, disease control, and treatment groups. Mice were treated with Nicotine-derived nitrosamine ketone (NNK) (10 mg/kg), pioglitazone (10 & 20 mg/kg) and celecoxib (25 & 50 mg/kg). Weekly body weight, food intake, mean survival time & % increased life span were determined. Tumor weight and histopathological analysis were performed at the end of the study. **Results.** The significant tumor reducing potential of pioglitazone combined with celecoxib was observed ($p < 0.05$). The treatment groups (treated with pioglitazone and celecoxib) showed a remarkable decrease in lung tumor weight, improved life span and mean survival time ($p < 0.05$). Histopathological studies confirm that treatment groups (treated with pioglitazone and celecoxib) reframed the lung architecture compared to disease control. **Conclusion.** Preliminary results revealed that pioglitazone adjunct with celecoxib may be an effective chemopreventive agent against NNK induce NSCLC.

Keywords Celecoxib · Pioglitazone · Mice · Lung tumorigenesis · Non-small cell lung cancer

Introduction

Lung cancer is one of the major malignancies, affecting more than 2.1 million people every year worldwide [1]. Based on the cellular architecture, there are two types of lung cancer, i.e., non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC accounts for about 85% of total lung cancer cases [2, 3]. Chemotherapeutics such as Docetaxel, Gemcitabine, Vinorelbine, Etoposide, Paclitaxel, Cisplatin and Carboplatin used in the treatment of NSCLC associate

with severe side effects such as bone marrow suppression, cardiotoxicity, neurotoxicity, nephrotoxicity, and ototoxicity [4–6]. In NSCLC, mutations in KRAS, ALK, and PTEN genes cause abnormal cell proliferation, angiogenesis, and metastasis. In general, these mutations promote activation of RAS/RAF/MEK, PI3K/Akt, and mTOR, leading to NF- κ B mediated NSCLC proliferation, metastasis, angiogenesis, and resistance to apoptosis [7–9].

Peroxisome proliferator-activated receptor- γ (PPAR- γ) is a ligand-activated transcription factor that belongs to nuclear receptor superfamily. In addition to its metabolic role, it also regulates cellular proliferation, angiogenesis, and metastasis [10–12]. PPAR- γ agonist, pioglitazone, used in the treatment of type 2 diabetes inhibits cancer cell proliferation, invasion, migration, and resistance to apoptosis by downregulating the gene expression of NF- κ B and, hence, interferes with carcinogenic cell pathways such as RAS/RAF/MEK and mTOR.

✉ Praveen T. Krishnamurthy
praveentk7812@gmail.com; praveentk@jssuni.edu.in

¹ Department of Pharmacology, JSS College of Pharmacy
(JSS Academy of Higher Education & Research) Rocklands,
Ooty, The Nilgiris, Tamil Nadu 643001, India

(Fig. 1) [13–18]. Although NSCLCs overexpress PPAR- γ , due to a limited activation by endogenous ligands (such as 15-deoxy- Δ 12,14 prostaglandin J2), its beneficial anticancer effects are curtailed [19–21]. Activation of PPAR- γ , using exogenous ligands, therefore, can significantly benefit in NSCLC.

NF- κ B mediated upregulation of cyclooxygenase-2 (COX-2) enzyme expression is well established in breast, lung, colon, cervical, and prostate cancers [22–27]. Recent studies show that overexpressed COX-2 in cancer cells can indirectly promote tumor metastasis and angiogenesis via thromboxane-A₂ mediated activation of the PI3K/Akt pathway (Fig. 1) [28]. Celecoxib, a selective COX-2 inhibitor, has shown the ability to reduce tumor metastasis by inhibiting the epithelial-mesenchymal transition (EMT) process in oral, breast, and gastric cancers [27–32]. In this study, we investigate the anti-tumor benefits of pioglitazone (PGZ), either alone or combined with celecoxib (CXB) in nicotine nitrosamine ketone (NNK) induced non-small cell lung cancer model of mice (Fig. 1).

Materials and methods

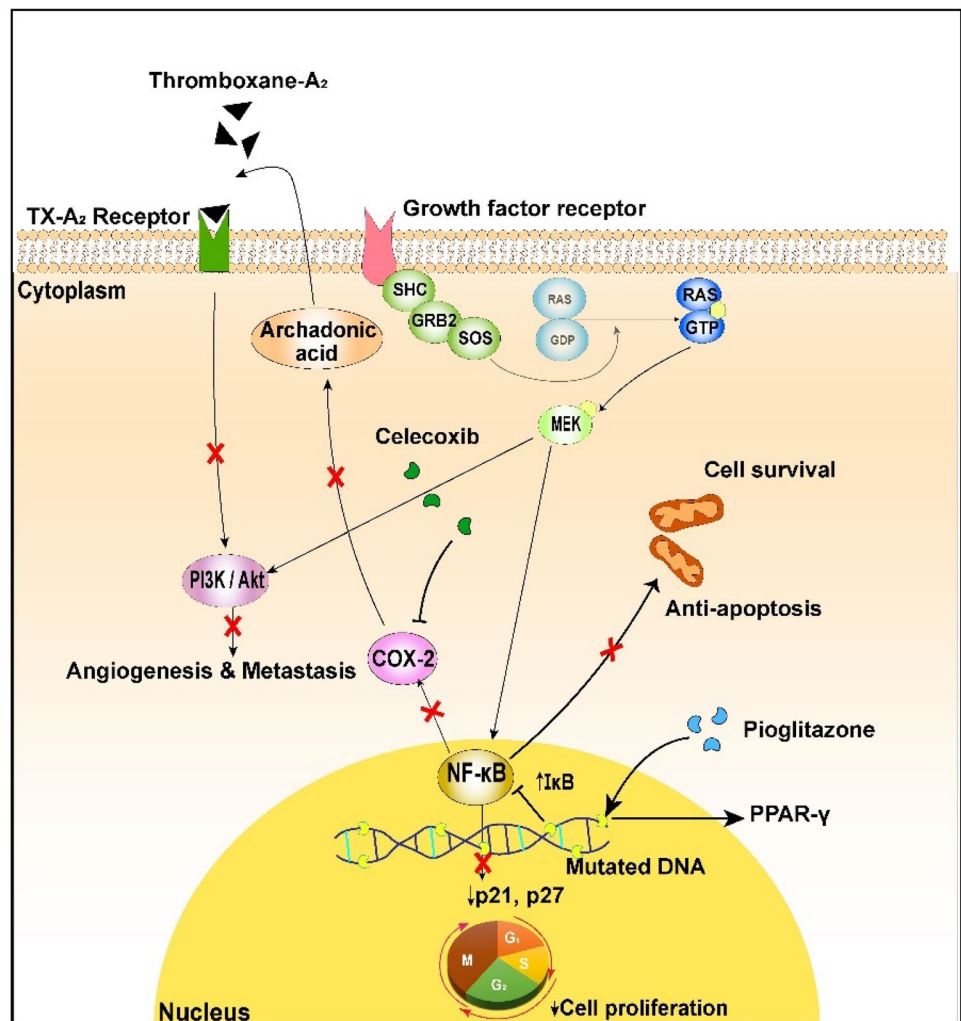
Reagents and chemicals

Nicotine nitrosamine ketone (NNK), pioglitazone (5-{4-[2-(5-ethylpyridin-2-yl) ethoxy]benzyl}-1,3-thiazolidine-2,4-dione), Celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzene-sulfonamide) were purchased from Sigma Chemical Co. Ltd., Bengaluru, Karnataka. All the other reagents (purity above 98%) were purchased from Himedia Co. Ltd., Bengaluru, Karnataka.

Animals

Sixty male Balb/c mice were housed in standard laboratory conditions (22 ± 2 °C, light: dark cycle of 12:12 h; Relative humidity: 30–70%) with normal pellet diet (M/s. Amrit feeds Ltd., Bengaluru, India) and water ad libitum. The experiment was performed with prior approval

Fig. 1 Mechanism of anticancer effects of pioglitazone and celecoxib: RAS/RAF/MEK, PI3K/Akt, and mTOR mediated activation of NF- κ B (not shown) increases the expression of in CDK blockers (p21, p27) and COX-2, which lead to NSCLC proliferation, metastasis, angiogenesis and resistance to apoptosis. PPAR- γ activation by pioglitazone decreases NF- κ B expression and hence inhibits NF- κ B mediated activation of carcinogenic pathways. Celecoxib, by inhibiting the COX-2 enzyme, can block NF- κ B mediated angiogenesis and metastasis



from the Institutional Animal Ethics Committee (IAEC) (Approval No. JSSCP/OT/M.PHARM/05/18–19).

Preparation of test solutions

A stock solution of Nicotine nitrosamine ketone (NNK) was prepared in sterile saline (1 mg/ml) and administered at a dose volume of 10 ml/kg body weight intratracheally to achieve 10 mg/kg dose.

Celecoxib and Pioglitazone were prepared as a suspension in CMC (0.5%) at a stock solution concentration of 1 & 2 mg/ml. These stock solutions were administered orally at a dose volume of 10 ml/kg body weight to achieve 10 & 20 mg/kg dose, respectively.

In vivo anticancer study

Induction of NSCLC in mice

NSCLC was induced in mice by intratracheal instillation of NNK (10 mg/kg). Intraperitoneal ketamine (80 mg/kg) & xylazine (10 mg/kg) cocktail was used to anesthetize mice. The hair around the neck region was depilated using depilatories (cosmetic depilatory) and cleansed with 70% alcohol. A 10 mm incision was made on the skin above the trachea to expose it. A 0.2 ml of prepared NNK solution was injected into the trachea using a 29G needle (Fig. 2). After NNK installation, the incision was stitched, and the surgical area was covered with beclomethasone ointment.

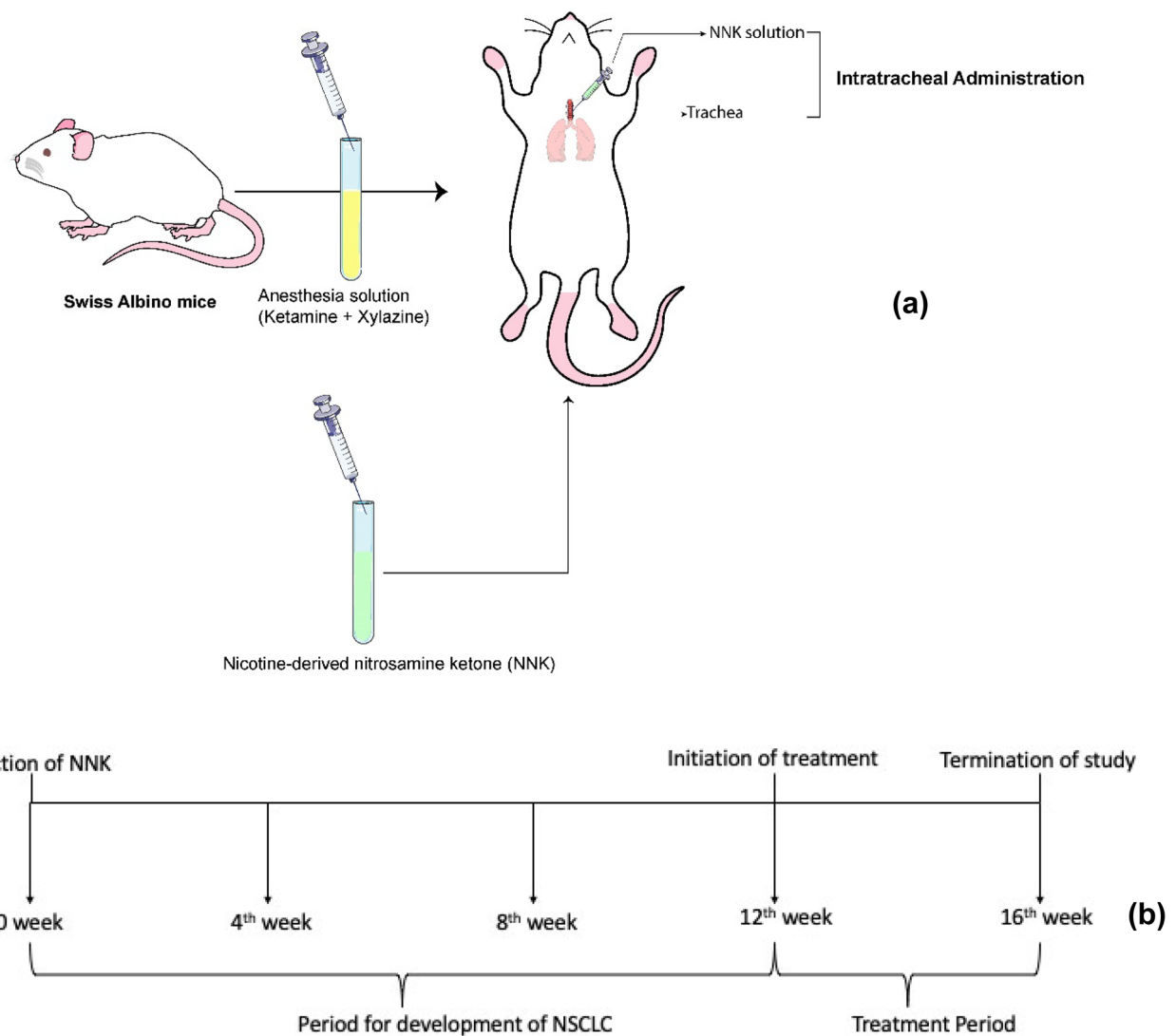


Fig. 2 (a) Intratracheal administration of Nicotine-derived nitrosamine ketone (NNK) via trachea (b) Anticancer study schedule

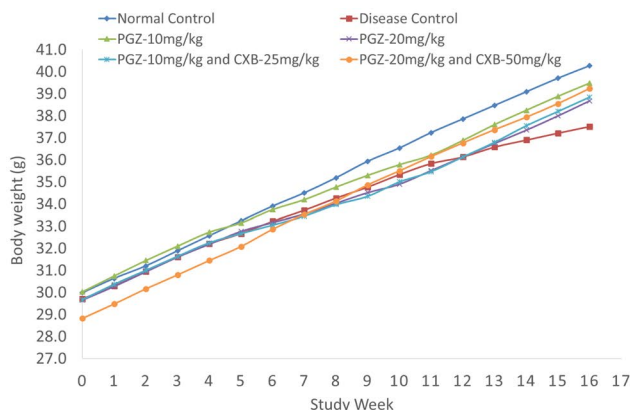
All the animals receive a single dose of levofloxacin (40 mg/kg, *i.v.*) to prevent any post-surgical infections. A set of ten animals were subjected to sham surgery, where the animals received sterile saline instead of NNK solution.

Animal grouping and treatment

Four weeks after NNK induction, animals except the sham control were divided into 6 groups of 10 each. Group 1 & 2 received distilled water, 10 ml/kg, *p.o.*, and serve as sham control and disease control, respectively. Group-3 & 4 received PGZ at a dose of 10 & 20 mg/kg, *p.o.*, respectively. Group-5 received a low dose of PGZ (10 mg/kg *p.o.*) & CXB (25 mg/kg *p.o.*). Group-6 received a high dose of PGZ (20 mg/kg, *p.o.*) & CXB (50 mg/kg, *p.o.*). In this study design, we did not consider a separate group for CXB alone treatment as it is used only as an adjuvant to PGZ. All the animals received the assigned treatment for a period of 12-weeks (starting from 4th week to 16th week) (Fig. 2b).

During the study period, all the animals were observed and recorded for abnormal clinical signs, mortality, and weekly body weight changes. The lung tissue was isolated from the animals, which are moribund or died during the study period. At the end of the study (at 33 weeks), the surviving animals were culled using deep isoflurane anesthesia to isolate the lung tissue. The tissue was washed with saline, blotted, and weighed before fixing in neutral buffered formalin (10% v/v) for histopathological analysis. From the survival data, the percentage increase in lifespan (% ILS) was calculated using the formulae.

$$\%ILS = \left(\frac{\text{Mean survival time of treatment group}}{\text{Mean survival time of disease control group}} - 1 \right) \times 100$$



Histopathological analysis

The lung tissue was fixed in neutral buffered formalin (10% v/v) processed and embedded in paraffin. Paraffin sections of 4 μm thick were prepared and stained with hematoxylin and eosin for histopathological examination.

Statistical analysis

All the data were represented as mean ± SD and analyzed by using one-way ANOVA followed by Dunnett's multiple comparison test (Graph Pad Prism, V.7, Graph Pad Software Inc., La Jolla, USA). $p < 0.05$ were considered significant.

Results

Effect of PGZ and CXB on body weight and net bodyweight change

The results are given in Fig. 3. Group-2, disease control, treated with NNK (10 mg/kg) alone show a significant decrease in weekly body weight from Week-10 onwards when compared to Group-1, normal control ($p < 0.05$). Group-3 treated with PGZ low dose (10 mg/kg, *p.o.*), Group-6 treated with PGZ & CXB high dose (20 & 50 mg/kg, *p.o.*, respectively) and Group-5 treated with PGZ & CXB low dose (10 & 25 mg/kg, *p.o.*, respectively) show a significant effect against NNK induced changes in body weight (Group-2, disease control) from Week 14, 15, and 16 onwards, respectively ($p < 0.05$). Other groups show only a non-significant effect ($p > 0.05$). The weekly net body weight change (a measure of change in body weight from week 0) data shows that Group-2, disease control, show a significant decrease in weekly body weight from Week-7 onwards when

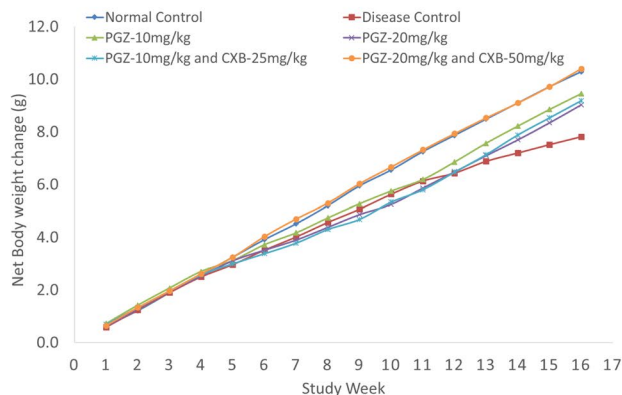
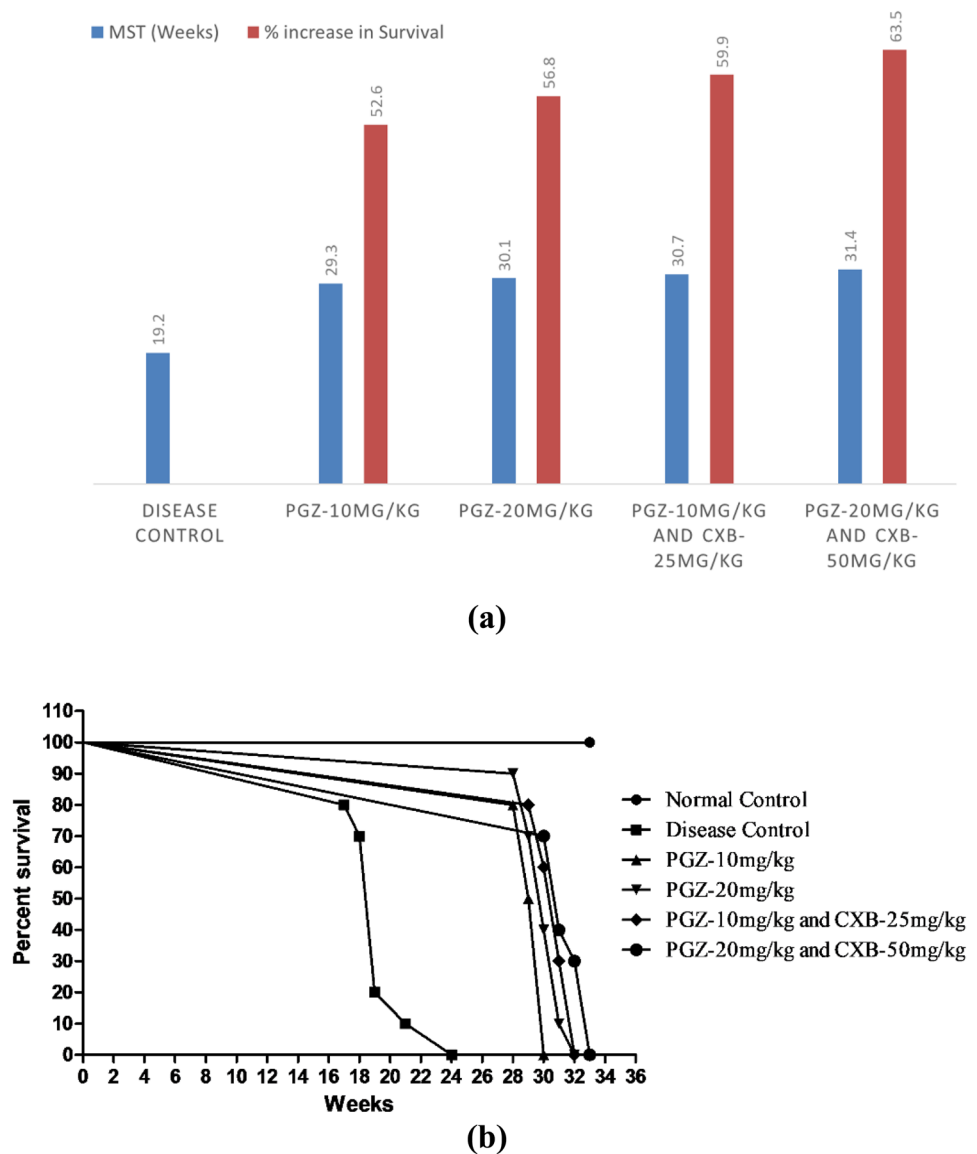


Fig. 3 Effect of PGZ and CXB on body weight and net bodyweight change in mice induced with NSCLC

Fig. 4 (a) Effect of PGZ and CXB on MST and %ILS in mice induced with NSCLC (b) Effect of PGZ and CXB on percentage survival in mice induced with NSCLC



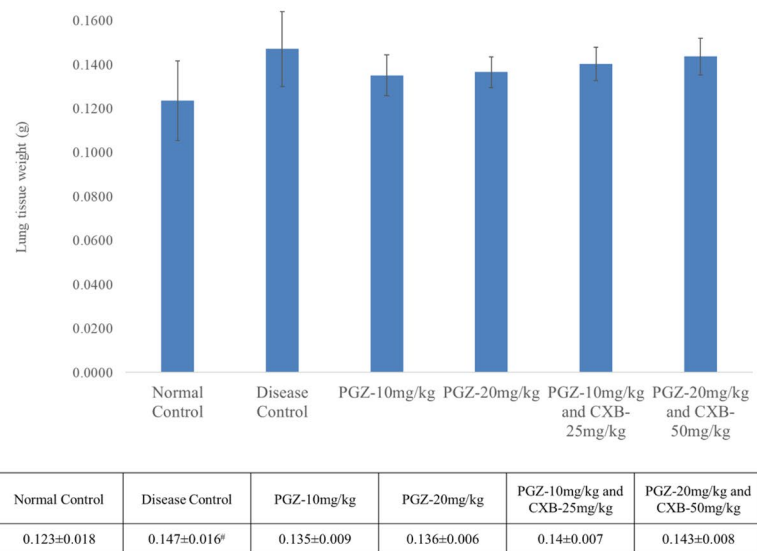
compared to Group-1, normal control ($p < 0.05$). Group-3 (PGZ-10 mg/kg, *p.o.*), Group-4 (PGZ-20 mg/kg, *p.o.*), Group-5 (PGZ & CXB 10 & 25 mg/kg, *p.o.*, respectively) and Group-6 (PGZ & CXB 20 & 50 mg/kg, *p.o.*, respectively) treatment groups significantly prevented the NNK effects on body weight from Week-14, 16, 15 and 8 onwards, respectively.

Effect of PGZ and CXB on percentage survival, mean survival time (MST) and % increase in lifespan (% ILS)

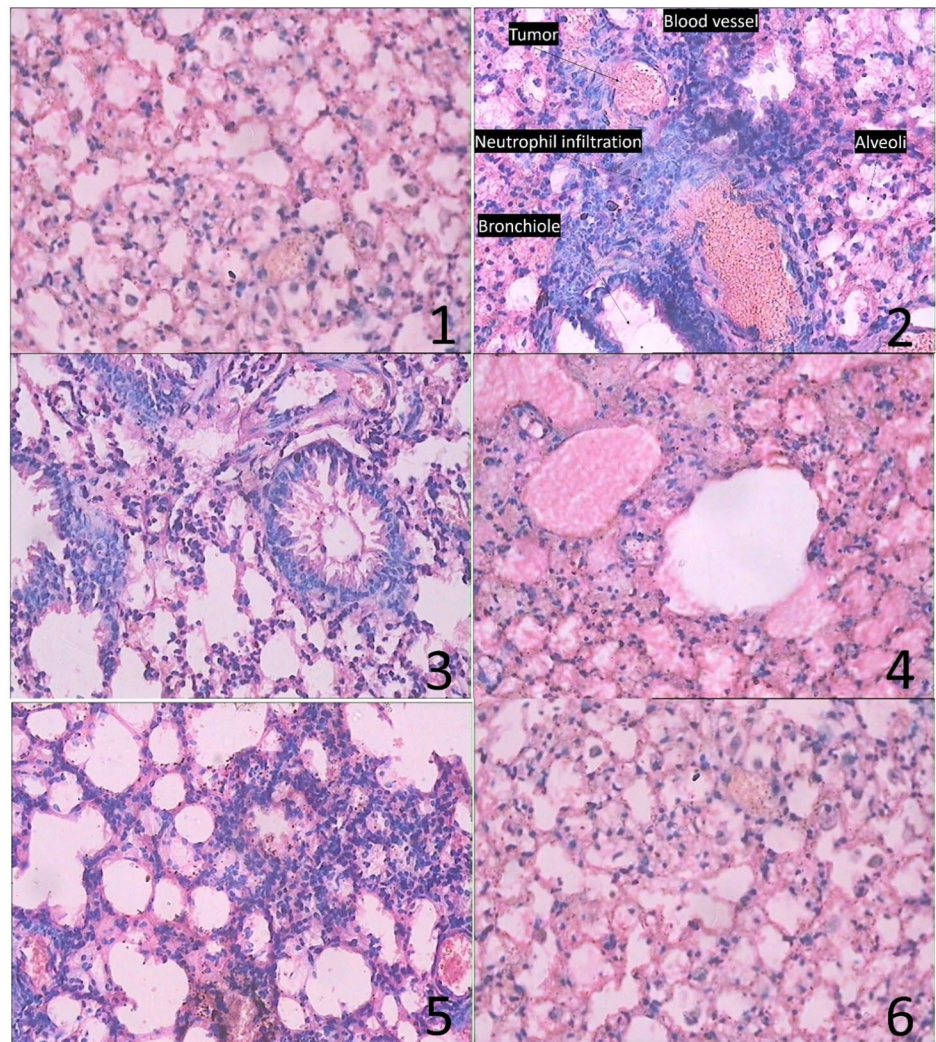
The results are given in Fig. 4a, b. Group-2, disease control, treated with NNK (10 mg/kg) alone shows a MST of 19.2 weeks. Group 3 & 4 treated with PGZ at a dose of 10 & 20 mg/kg, *p.o.*, increase the MST to 29.3 & 30.1 weeks

and % ILS to 52.6 & 56.8%, respectively, when compared to Group-2, disease control. Group 5 & 6 treated with PGZ & CXB (10 & 25 mg/kg, *p.o.*, and 20 & 50 mg/kg, *p.o.*, respectively) show a further increase in MST to 30.7 & 31.4 weeks and %ILS to 59.9 & 63.5%, respectively, when compared to Group-2, disease control. These results, therefore, may suggest combining CXB with PGZ may have beneficial effects in the treatment of NSCLC. The percentage survival graph shows that in Group-2, disease control, all the animals have died by 24 weeks, and at the same point in treatment groups, all the animals have survived. The highest rate of survival was observed in Group-6, treated with PGZ & CXB (20 & 50 mg/kg, *p.o.*, respectively), where 30% of the animals have survived at week-32 (Fig. 4b).

Fig. 5 (a) Effect of PGZ and CXB on lung tissue weight in mice induced with NSCLC (Values are mean \pm SD, n=10, #: p < 0.05 when compared to Group-1, Normal control. *: p < 0.05 when compared to Group-2, Disease control) **(b)** Lung histopathology of mice induced with NSCLC: 1-Sham control (Distilled water-10 ml/kg, p.o.); 2- Disease control (Distilled water 10mg/kg p.o.); 3-PGZ (10 mg/kg p.o.); 4- PGZ (20 mg/kg p.o.), 5- PGZ (10 mg/kg p.o.) and CXB (25 mg/kg p.o.); and 6- PGZ (20 mg/kg p.o.) and CXB (50 mg/kg p.o.) (H&E, 40X)



(a)



(b)

Effect of PGZ and CXB on lung tissue weight

The lung tissue weights are recorded to indirectly measure tumor growth. The results are given in Fig. 5a. Group-2, disease control, treated with NNK (10 mg/kg) alone shows a significant increase in lung tissue weight when compared to Group-1, normal control ($p < 0.05$). All the treatment groups show only a non-significant effect against the NNK induced changes in lung tissue weight ($p < 0.05$).

Histopathological studies

The histopathological analysis of NNK-induced NSCLC in mice was shown in Fig. 5b. Group 1 & 2 received distilled water, 10 ml/kg, *p.o.*, and serve as sham control and disease control, respectively. Group-3 & 4 received PGZ at a dose of 10 & 20 mg/kg, *p.o.*, respectively. Group-5 received a low dose of PGZ (10 mg/kg *p.o.*) & CXB (25 mg/kg *p.o.*). Group-6 received a high dose of PGZ (20 mg/kg, *p.o.*) & CXB (50 mg/kg, *p.o.*). In this study design, we did not consider a separate group for CXB alone treatment as it is used only as an adjuvant to PGZ.

Group-1, sham control treated with distilled water, 10 ml/kg, *p.o.*, show normal lung architecture with distinct alveolar space. Group-2, disease control induced with NSCLC with NNK shows significant abnormalities in lung architecture indicative of proliferating cells obliterating the alveolar space with a significant number of adenocarcinoma and neutrophil infiltration. Group-3 & 4 treated with PGZ at a dose of 10 & 20 mg/kg, *p.o.*, respectively, show significant prevention of NNK induced changes in lung histology. Group-5 & 6 treated with PGZ (10 mg/kg *p.o.*) & CXB (25 mg/kg *p.o.*) and PGZ (20 mg/kg *p.o.*) & CXB (50 mg/kg *p.o.*), respectively, show dose-dependent prevention NNK induced changes in lung histology. The highest anticancer activity was observed with Group-6 treated with a high dose of PGZ and CXB.

Discussion

The main aim of this investigation was to evaluate the chemo-preventive effects of PGZ and CXB against the chemically induced NSCLC. Our results show the dramatic suppression of lung carcinogenesis of COX-2 inhibition and PPAR- γ activation. It is noteworthy that the administration of PGZ and CXB did not produce any toxic side effects such as ulceration, edemas, etc. These results support the earlier findings suggesting the potential of PPAR- γ agonists and COX-2 antagonists as chemo-preventive against NSCLC and underscore the need for a thorough investigation on PGZ and CXB as a potentially effective approach. It is also important to note

that the anti-tumor effects of PGZ and CXB alone have been observed against other types of cancer [11, 29–31, 33]. The possibility, therefore, exists that PGZ combined with CXB may become a general chemopreventive agent against many malignancies.

The exact mechanism of action by which PGZ and CXB inhibit lung carcinogenesis remains to be clarified. Our working hypothesis is that NSCLC is triggered by activation of α_7 Ach receptors by NNK, which upregulates the NF κ B, therefore increasing the carcinogenesis and COX-2 levels in the lungs [34]. The aberrant activation of NF κ B could, therefore, result in (a) increase tumor progression (b) inhibit the anti-apoptotic proteins from mitochondria (c) and upregulate COX-2 levels which cause angiogenesis by stimulating VEGF via PGE₂ and metastasis by PI3K/Akt pathway via thromboxane mediated activation. Therefore, blocking of NF κ B and COX-2 using suitable agents could modulate the critical initiation and promotion of lung carcinogenesis [34]. Previous studies have reported that PPAR γ is important in cancer cell growth and PPAR γ agonists could inhibit cell growth and induce apoptosis in different malignancies including breast, colon, liver, lung, etc. [15, 33, 35–37]. This tumor inhibition is reported to be due to PPAR γ mediated p65 degradation, thus leading to inhibition of NF κ B [15]. In a study, M-Y. Li and colleagues reported that pioglitazone prevented smoking carcinogen (NNK)-induced lung tumors by inducing apoptosis by up-regulating PPAR γ expression [38]. COX-2 has also been reported to promote tumorigenesis as it is revealed to be involved in fundamental processes to tumor formation, including invasion, angiogenesis, and metastasis [22, 39–42]. In a previous study, Nathalie Rioux et al. reduced NNK-induced lung tumorigenesis in a dose-dependent manner by using NS-398, a selective COX-2 inhibitor [43]. Based on the above evidence, inhibition of COX-2 and activation of PPAR γ may be useful in treating NNK induced NSCLC.

Conclusion

In this work, we explored the role of combined administration of PGZ and CXB NNK induced NSCLC in swiss albino mice. The degree of inhibition was more pronounced with a combination of PGZ and CXB than with PGZ alone. The possible mechanism may include an increase in PPAR γ transcription factors, inhibition of NF κ B and COX-2 mRNA at the nuclear level, and COX-2 inhibition at cytoplasmic level, therefore decreasing tumor proliferation, survival, angiogenesis, and metastasis. These results reflect that PGZ combined with CXB may be an effective chemo-preventive agent against NNK induce NSCLC.

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Author contributions PTK and AVVVRK generated hypothesis. PTK helped in conceptualization, supervision, validation and proof reading. AVVVRK has conceptualized, carried out the work, data analysis, writing-original draft; GKK validated and proof-read the manuscript.

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Data availability All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval The experiment was performed with prior approval from the Institutional Animal Ethics Committee (IAEC) (Approval No. JSSCP/OT/M.PHARM/05/18–19).

Conflicts of interest The authors do not have any conflicts of interest.

References

- Siegel RL, Miller KD, Jemal A (2020) Cancer statistics, 2020. *CA Cancer J Clin* 70:7–30. <https://doi.org/10.3322/caac.21590>
- Khuder SA (2001) Effect of cigarette smoking on major histological types of lung cancer: a meta-analysis. *Lung Cancer* 31:139–148. [https://doi.org/10.1016/S0169-5002\(00\)00181-1](https://doi.org/10.1016/S0169-5002(00)00181-1)
- Morabia A, Wynder EL (1991) Cigarette smoking and lung cancer cell types. *Cancer* 68:2074–2078. [https://doi.org/10.1002/1097-0142\(19911101\)68:9%3c2074::AID-CNCR2820680939%3e3.0.CO;2-X](https://doi.org/10.1002/1097-0142(19911101)68:9%3c2074::AID-CNCR2820680939%3e3.0.CO;2-X)
- Rubins J (2001) Weekly paclitaxel in advanced non-small cell lung cancer. *Semin Oncol* 28:10–13. [https://doi.org/10.1016/S0093-7754\(01\)90053-3](https://doi.org/10.1016/S0093-7754(01)90053-3)
- Ricci S (2000) Gemcitabine monotherapy in elderly patients with advanced non-small cell lung cancer A multicenter phase II study. *Lung Cancer* 27:75–80. [https://doi.org/10.1016/S0169-5002\(99\)00098-7](https://doi.org/10.1016/S0169-5002(99)00098-7)
- Florea A-M, Büsselberg D (2011) Cisplatin as an Anti-Tumor Drug: Cellular Mechanisms of Activity, Drug Resistance and Induced Side Effects. *Cancers* 3:1351–1371. <https://doi.org/10.3390/cancers3011351>
- Jin G, Kim MJ, Jeon H-S et al (2010) PTEN mutations and relationship to EGFR, ERBB2, KRAS, and TP53 mutations in non-small cell lung cancers. *Lung Cancer* 69:279–283. <https://doi.org/10.1016/j.lungcan.2009.11.012>
- Riely GJ, Marks J, Pao W (2009) KRAS Mutations in Non-Small Cell Lung Cancer. *Proc Am Thorac Soc* 6:201–205. <https://doi.org/10.1513/pats.200809-107LC>
- Gridelli C, Peters S, Sgambato A et al (2014) ALK inhibitors in the treatment of advanced NSCLC. *Cancer Treat Rev* 40:300–306. <https://doi.org/10.1016/j.ctrv.2013.07.002>
- Lehrke M, Lazar MA (2005) The Many Faces of PPAR γ . *Cell* 123:993–999. <https://doi.org/10.1016/j.cell.2005.11.026>
- Elnemr A, Ohta T, Iwata K et al (2000) PPAR γ ligand (thiazolidinedione) induces growth arrest and differentiation markers of human pancreatic cancer cells. *Int J Oncol*. <https://doi.org/10.3892/ijo.17.6.1157>
- Lecarpentier Y, Claes V, Vallée A, Hébert J-L (2017) Interactions between PPAR Gamma and the Canonical Wnt/Beta-Catenin Pathway in Type 2 Diabetes and Colon Cancer. *PPAR Res* 2017:1–9. <https://doi.org/10.1155/2017/5879090>
- Straus DS, Pascual G, Li M et al (2000) 15-Deoxy-Delta 12,14-prostaglandin J2 inhibits multiple steps in the NF-kappa B signaling pathway. *Proc Natl Acad Sci* 97:4844–4849. <https://doi.org/10.1073/pnas.97.9.4844>
- Chen F, Wang M, O'Connor JP et al (2003) Phosphorylation of PPAR γ via active ERK1/2 leads to its physical association with p65 and inhibition of NF- κ B. *J Cell Biochem* 90:732–744. <https://doi.org/10.1002/jcb.10668>
- Wang F, Liu Y, Bi Z (2017) Pioglitazone inhibits growth of human retinoblastoma cells via regulation of NF- κ B inflammation signals. *J Recept Signal Transduct* 37:94–99. <https://doi.org/10.3109/10799893.2016.1171341>
- Ciaramella V, Sasso FC, Di Liello R et al (2019) Activity and molecular targets of pioglitazone via blockade of proliferation, invasiveness and bioenergetics in human NSCLC. *J Exp Clin Cancer Res* 38:178. <https://doi.org/10.1186/s13046-019-1176-1>
- Fan P, Abderrahman B, Chai TS et al (2018) Targeting Peroxisome Proliferator-Activated Receptor γ to Increase Estrogen-Induced Apoptosis in Estrogen-Deprived Breast Cancer Cells. *Mol Cancer Ther* 17:2732–2745. <https://doi.org/10.1158/1535-7163.MCT-18-0088>
- Camp HS, Tafuri SR (1997) Regulation of Peroxisome Proliferator-activated Receptor γ Activity by Mitogen-activated Protein Kinase. *J Biol Chem* 272:10811–10816. <https://doi.org/10.1074/jbc.272.16.10811>
- Forman BM, Tontonoz P, Chen J et al (1995) 15-Deoxy- Δ 12,14-Prostaglandin J2 is a ligand for the adipocyte determination factor PPAR γ . *Cell* 83:803–812. [https://doi.org/10.1016/0092-8674\(95\)90193-0](https://doi.org/10.1016/0092-8674(95)90193-0)
- Bell-Parikh LC, Ide T, Lawson JA et al (2003) Biosynthesis of 15-deoxy- Δ 12,14-PGJ2 and the ligation of PPAR γ . *J Clin Invest* 112:945–955. <https://doi.org/10.1172/JCI200318012>
- Kliwer SA, Lenhard JM, Willson TM et al (1995) A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. *Cell* 83:813–819. [https://doi.org/10.1016/0092-8674\(95\)90194-9](https://doi.org/10.1016/0092-8674(95)90194-9)
- Tsuji M, Kawano S, DuBois RN (1997) Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci U S A* 94:3336–3340
- Dohadwala M, Luo J, Zhu L et al (2001) Non-small Cell Lung Cancer Cyclooxygenase-2-dependent Invasion Is Mediated by CD44. *J Biol Chem* 276:20809–20812. <https://doi.org/10.1074/jbc.C100140200>
- Lee L, Pan C, Cheng C et al (2001) Expression of cyclooxygenase-2 in prostate adenocarcinoma and benign prostatic hyperplasia. *Anticancer Res* 21:1291–1294
- Wolff H, Saukkonen K, Anttila S et al (1998) Expression of Cyclooxygenase-2 in Human Lung Carcinoma. *Cancer Res* 58:4997
- Kulkarni S, Rader JS, Zhang F et al (2001) Cyclooxygenase-2 Is Overexpressed in Human Cervical Cancer. *Clin Cancer Res* 7:429
- Grösch S, Tegeder I, Niederberger E et al (2001) COX-2 independent induction of cell cycle arrest and apoptosis in colon cancer cells by the selective COX-2 inhibitor celecoxib. *FASEB J* 15:1–22. <https://doi.org/10.1096/fj.01-0299fj>
- Hsu A-L, Ching T-T, Wang D-S et al (2000) The Cyclooxygenase-2 Inhibitor Celecoxib Induces Apoptosis by Blocking Akt Activation

- in Human Prostate Cancer Cells Independently of Bcl-2. *J Biol Chem* 275:11397–11403. <https://doi.org/10.1074/jbc.275.15.11397>
29. Hu PJ (2004) Chemoprevention of gastric cancer by celecoxib in rats. *Gut* 53:195–200. <https://doi.org/10.1136/gut.2003.021477>
 30. Harris RE, Alshafie GA, Abou-Issa H, Seibert K (2000) Chemoprevention of breast cancer in rats by celecoxib, a cyclooxygenase 2 inhibitor. *Cancer Res* 60:2101–2103
 31. Reddy BS, Hirose Y, Lubet R et al (2000) Chemoprevention of Colon Cancer by Specific Cyclooxygenase-2 Inhibitor, Celecoxib, Administered during Different Stages of Carcinogenesis. *Cancer Res* 60:293
 32. Chiang S-L, Velmurugan BK, Chung C-M et al (2017) Preventive effect of celecoxib use against cancer progression and occurrence of oral squamous cell carcinoma. *Sci Rep* 7:6235. <https://doi.org/10.1038/s41598-017-06673-3>
 33. Inoue K, Kawahito Y, Tsubouchi Y et al (2001) Expression of peroxisome proliferator-activated receptor (PPAR)-gamma in human lung cancer. *Anticancer Res* 21:2471–2476
 34. Ravi Kiran Ammu VVV, Garikapati KK, Krishnamurthy PT et al (2019) Possible role of PPAR- γ and COX-2 receptor modulators in the treatment of Non-Small Cell lung carcinoma. *Med Hypotheses* 124:98–100. <https://doi.org/10.1016/j.mehy.2019.02.024>
 35. Hase T, Yoshimura R, Mitsuhashi M et al (2002) Expression of peroxisome proliferator-activated receptors in human testicular cancer and growth inhibition by its agonists. *Urology* 60:542–547. [https://doi.org/10.1016/S0090-4295\(02\)01747-8](https://doi.org/10.1016/S0090-4295(02)01747-8)
 36. Eibl G, Wente MN, Reber HA, Hines OJ (2001) Peroxisome Proliferator-Activated Receptor γ Induces Pancreatic Cancer Cell Apoptosis. *Biochem Biophys Res Commun* 287:522–529. <https://doi.org/10.1006/bbrc.2001.5619>
 37. DuBois R (1998) The nuclear eicosanoid receptor, PPARgamma, is aberrantly expressed in colonic cancers. *Carcinogenesis* 19:49–53. <https://doi.org/10.1093/carcin/19.1.49>
 38. Li M-Y, Kong AWY, Yuan H et al (2012) Pioglitazone prevents smoking carcinogen-induced lung tumor development in mice. *Curr Cancer Drug Targets* 12:597–606
 39. Vane JR, Bakhle YS, Botting RM (1998) CYCLOOXYGENASES 1 AND 2. *Annu Rev Pharmacol Toxicol* 38:97–120. <https://doi.org/10.1146/annurev.pharmtox.38.1.97>
 40. Dubois RN, Abramson SB, Crofford L et al (1998) Cyclooxygenase in biology and disease. *FASEB J Off Publ Fed Am Soc Exp Biol* 12:1063–1073
 41. Tsujii M, DuBois RN (1995) Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* 83:493–501
 42. Tsujii M, Kawano S, Tsuji S et al (1998) Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell* 93:705–716
 43. Rioux N, Castonguay A Prevention of NNK-induced Lung Tumorigenesis in A/J Mice by Acetylsalicylic Acid and NS-398. 8

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