Am J Transl Res 2023;15(11):6649-6659
www.ajtr.org /ISSN:1943-8141/AJTR0151713

Review Article

Soluble epoxide hydrolase inhibitors for smoking-associated inflammatory lung diseases and chronic obstructive pulmonary disease: a meta-analytical systematic review of preclinical and clinical studies

Alberto G Borges Felipe[1*], Camila B Miguel[1,2*], Ana P Lazarin Bernardes[2*], Rodrigo Perissinotto[2], Carlos A Trindade-da-Silva[3,5], Melissa C Martins-de-Abreu[2], Jamil Miguel-Neto[2], Wellington F Rodrigues[1,2,4], Carlo J Freire Oliveira[1,4]

[1]Postgraduate Program in Health Sciences, Federal University of Triângulo Mineiro, Uberaba, MG, Brazil; [2]Course of Medicine at The University Center of Mineiros, Mineiros, GO, Brazil; [3]Institute and Research Center São Leopoldo Mandic, São Leopoldo Mandic Faculty, Campinas, SP, Brazil; [4]Postgraduate Program in Tropical Medicine and Infectious Diseases, Federal University of Triângulo Mineiro, Uberaba, MG, Brazil; [5]Instituto Nacional de Ensino Superior e Pós-Graduação Padre Gervásio., Pouso Alegre, MG, Brazil. [*]Equal contributors.

Received June 11, 2023; Accepted October 30, 2023; Epub November 15, 2023; Published November 30, 2023

Abstract: An evaluation of the inflammatory enzymatic interactions related to pulmonary function can help identify biomarkers for interventions or prophylactic measures to improve patient prognosis. This study aimed to determine the effect of epoxide hydrolase inhibition by GSK2256294 in different pulmonary inflammation models. A secondary search was performed using Medline/PubMed, Web of Science, SciELO, Cochrane Library, Embase, Academic Google, and gray literature by two independent reviewers, who analyzed the methodological quality and consistency of the data. Different variables were compared using a meta-analysis. A total of 86 studies were found, 4 of which were selected from the gray literature. Based on the eligibility criteria, two clinical and one preclinical studies were evaluated. GSK2256294 inhibited the soluble epoxide hydrolase enzyme in both clinical and preclinical models, exhibiting greater effectiveness in clinical studies and contributing to the anti-inflammatory activity mediated by the eicosatrienoic pathway by reducing the levels of dihydroxyeicosatrienoic acids and leukotoxin-diol. Overall, GSK2256294 was identified as a promising drug for controlling the deleterious manifestations of lung inflammation. Further clinical and preclinical studies are required to ensure consistency among the evidence and identify other biological activities mediated by GSK2256294.

Keywords: Enzymes, lung function, inflammation, clinical study, preclinical study

Introduction

Smoking is a risk factor for chronic diseases such as cerebrovascular disease; malignant neoplasms of the lungs, mouth, larynx, and pharynx; acute coronary disease (angina and infarction); and chronic obstructive pulmonary disease (COPD). Globally, tobacco use causes over seven million deaths per year. Besides smoking, several other pathophysiological changes are associated with inflammatory lung diseases, including central obesity and metabolic syndromes [1], leading to chronic inflammation and endothelial dysfunction [2].

Inflammatory lung diseases involve several modulation pathways, including T-helper type 2 (Th2) responses observed in hypersensitivity-mediated diseases and several other Th1, Th17, and Th22 profiles, with adaptive and innate immune cells as participants [3-5]. Although primary factors initiate the disease, diverse enzymatic activities mediate different responses [6, 7].

In inflammatory lung diseases, airflow restriction caused by an inflammatory response to inhaled toxins, often cigarette smoking, causes oxidative stress, chronic hypoxia, arterial stiff-

ness, endothelial dysfunction, vasodilator and vasoconstrictor substance imbalance, alterations in the pro-inflammatory cytokine levels, inflammation, and senescence of endothelial cell factors. Therefore, techniques such as venous occlusion plethysmography, forearm flow-mediated dilation, and biomarker analyses have been employed to assess endothelial and peripheral functions in individuals with pulmonary inflammation [8] and the ensuing alteration of the pulmonary vascular endothelium.

Epoxyeicosatrienoic acids (EETs) are hyperpolarizing factors derived from the endothelium from arachidonic acid metabolism by cytochrome P450 epoxygenases [9], which are factors that control vasomotor tone [10], inhibit nuclear factor-κB (NF-κB) signaling pathway activation via potassium channel modulation, and exhibit anti-apoptotic effects on endothelial cells and anti-inflammatory properties [11, 12]. In plants and animals, epoxide hydrolases convert epoxide diols into diols via hydrolysis. In mammals, soluble epoxide hydrolase (sEH) is responsible for the metabolism of arachidonic acid derivatives and EETs. sEH converts EETs to dihydroxy eicosatrienoic acids (DHETs) [13].

Increased sEH activity may indicate increased inflammation, as observed in patients with obesity who are smokers with coronary artery disease, where the sEH concentration was associated with the lowest EET/DHET ratios [2]. Additionally, EETs are produced in lung epithelial cells, and patients with COPD may experience loss of function due to high sEH activity [2, 14].

sEH is found in different cell types, including epithelial, endothelial, and pulmonary smooth muscle cells. sEH is also present in inflammatory cells, such as macrophages, eosinophils, neutrophils, and T cells, and in plasma with reduced activity [15, 16].

GSK2256294 is a potent and selective sEH inhibitor discovered using DNA-encoded Chemical Libraries based on a combination of oligonucleotides [17]. GSK2256294 can bind to the enzymatic catalytic site of sEH, inactivating its action and contributing to an increase in EET levels and a decrease in its conversion to DHETs and leukotoxin-diol [18, 19]. The increased EET levels owing to GSK2256294 are related to its vasodilatory properties, as this drug relaxes the blood vessels and promotes increased blood flow. EETs are also related to reduced expression of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin 1β (IL-1β), in several cells, including endothelial cells and immune system cells, such as macrophages and lymphocytes, thereby reducing inflammation. NF-kB activation inhibition via increased EET levels is an important mechanism in which the activation of nuclear factor kappa B (NF-κB), a key regulator of the expression of genes involved in inflammation, is inhibited [20, 21].

GSK2256294 has been tested in preclinical and randomized clinical trials and has been demonstrated to affect pulmonary and systemic endothelial function [13] and attenuate smoking-induced pulmonary inflammation. sEH inhibition prevents the initiation and maintenance of the pulmonary inflammatory response and promotes its resolution [2, 22, 23]. Steroidal and nonsteroidal anti-inflammatory drugs can be administered to inhibit enzymatic activity, thereby controlling inflammatory diseases [24, 25].

Evidence of GSK2256294 activity in the inhibition and resolution of pulmonary inflammatory responses has been published [2, 22, 23].

An experimental model revealed that sEH inhibition attenuates inflammation and emphysema secondary to smoking while improving metabolic syndrome symptoms and pulmonary functions [26]. Strategies, such as anti-inflammation, are widely used to inhibit enzymes that promote inflammation and tissue damage [25]. However, further studies on biomolecules with higher selectivity and efficacy are required. Therefore, in this study, we evaluated the efficacy of GSK2256294 and its effects on epoxide hydrolase inhibition by GSK2256294 in different pulmonary inflammation models.

Materials and methods

Ethical aspects

This secondary study did not violate any current legislation related to ethics in research on humans or animals.

Registration protocol

This study was registered in the Open Science Framework database (OSF Registers) under the doi, 10.17605/OSF.IO/PMHA8 and is structured according to the Preferred Reporting Items for Systematic Protocols (PRISMA 2020) [27].

Search strategy

Medline (PubMed), Web of Science, SciELO, Cochrane Library, Embase, Google Scholar, and gray literature databases were searched. Medical Subject Headings (MeSH) were used to define the descriptors. The following descriptors were used to select eligible studies: "N-((4-cyano-2-(trifluoromethyl)phenyl)methyl)-3-((4-methyl-6-(methylamino)-1,3,5-triazin-2-yl) amino)cyclohexanecarboxamide" and "Pulmonary Disease, Chronic Obstructive", with their respective entry terms (entry terms: GSK22-56294, Chronic Obstructive Lung Disease, Chronic Obstructive Pulmonary Diseases, COAD, COPD, Chronic Obstructive Airway Disease, Chronic Obstructive Pulmonary Disease, Airflow Obstruction, Chronic Airflow Obstruction, Chronic Airflow Obstruction, Chronic Airflow Obstruction). The Boolean operators "AND" and "OR" were used to aid the search. The search form was adapted based on the characteristics of each platform. Two researchers performed the search in a non-paired manner between August and September 2022.

Eligibility criteria

Clinical and preclinical studies, both *in vivo* or *in vitro*, that examined the relationship between lung parenchyma inflammation and enzymatic activity, irrespective of whether they were evaluated as developmental, exposure, or outcome variables, were eligible for inclusion in the analysis. All reports were accepted as publications in the databases, and no time restrictions were applied to the search for these articles.

Studies that reported arguments related to the theme but were not products of research designs, such as narrative reviews and studies unrelated to the theme, were excluded from the analysis. Studies indexed in PubMed over the last 10 years were also evaluated. The extracted data included characteristics of the studies and their primary approaches. Finally, data were evaluated and subjected to a qualitative assessment using tables by two independent reviewers. The data were then compiled, and conflicts were resolved by a third author.

Methodological and evidence quality

Two reviewers independently assessed the methodological quality of the included studies, and conflicting opinions were resolved via discussion. ROB 2.0 [28], which assesses the risk of bias in clinical studies, and SYRCLE [29], which assesses the risk of bias in animal studies, were employed for the analysis. SYRCLE contains the following assessment categories: selection, performance, detection, attrition, reporting, and other sources of bias. We applied 10 questions to the articles included in the systematic review, with responses of "YES" indicating a low risk of bias, "NO" indicating a high risk of bias, and "UNCERTAIN" indicating an uncertain risk of bias. Using this tool to calculate the total score for each study is not recommended [29].

Data analysis

Data were tabulated in Microsoft Excel, and RStudio was used for data analysis and visual display.

The General Package for Meta-Analyses version 4.9-5 was used to assess and compare the prevalence (metaprop), mean (metacont), and meta-regression (metareg). A "forest plot" was used to evaluate and represent data and generate a correlation chart for F(X). Study heterogeneity was assessed using the I^2 statistic from Cochran's Q statistic and the number J of analyzed studies. Based on the estimated heterogeneity, a random-effect model was implemented for meta-analysis [30-33].

Results

A total of 86 studies were retrieved from the databases using the established search key, four of which were selected from the gray literature. After applying the eligibility criteria, three studies were classified and analyzed (**Figure 1**).

By assessing the methodological quality of eligible studies, "some concerns" were found for

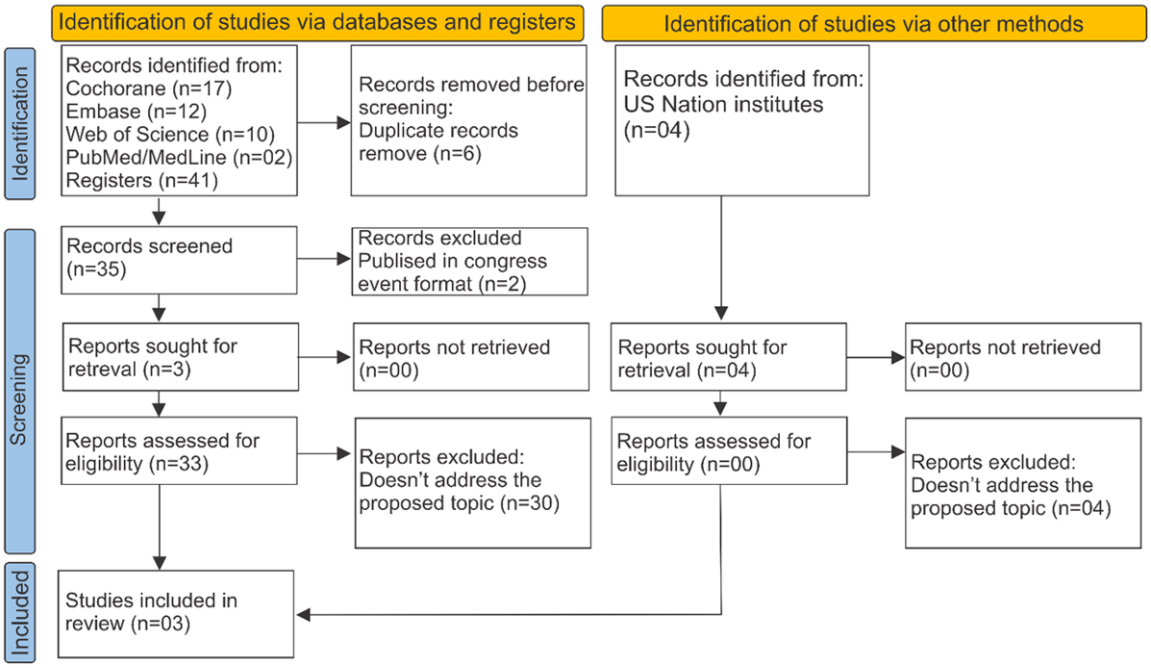

Figure 1. Flowchart of the selection of eligible studies for the systematic review per the eligibility criteria (PRISMA 2020).

clinical studies (Supplementary Table 1), and a moderate risk of bias was found for preclinical studies (Supplementary Table 2), per the recommendations of each applied tool (Robbis2 and SYRCLE).

The effects of GSK2256294 on sEH levels were verified using different models (**Figure 2**). When frequencies were grouped (I^2 = 96%), high heterogeneity was observed, with significant differences between the study models (P < 0.001). The greatest reduction was observed in an *in vitro* evaluation of human blood samples (93% reduction).

Experiments using rats revealed a 38% reduction in GSK2256294 activity. However, a high reduction rate was obtained using mice (61%) and rodents (50%).

After determining the inhibitory activities of the sEH enzyme using GSK2256294 in different pulmonary inflammation models, the effect of decreasing DHETS was evaluated and correlated with GSK2256294 concentrations in different models (clinical and preclinical) (**Figure 3**). A significant positive correlation was found in all models. When the models were grouped, a positive correlation of 0.76 was found. When the models were evaluated separately, an increase in the heterogeneity of DHES reduction values was observed in the clinical model (rho = 0.64). Notably, heterogeneity decreased while the correlation increased (rho = 0.92) in the preclinical model (**Figure 3**).

sEH participates in the conversion of leukotoxins to leukotoxin-diol, which exhibits inflammatory activity. The relationship between these two compounds enabled the evaluation of the enzymatic activity and inflammatory status of the evaluated model. Thus, the effects of the leukotoxin-to-leukotoxin-diol ratio in a pulmonary inflammation model treated with GSK-2256294 were verified (**Figure 4**). Pooling the effects of GSK2256294 on the leukotoxin-to-leukotoxin-diol ratio between clinical and preclinical models resulted in low heterogeneity (I^2 = 9%), with a mean ratio-increasing effect of 27% (CI = 0.35 to 2.18) (**Figure 4**).

The effect of GSK2256294 on bradykinin-associated blood flow was determined in a clinical study of lung injury in smokers (**Figure 5**). In eligible studies, blood flow was assessed following administration of 300 mg of bradykinin, either in the presence or absence of GSK-2256294. The relationships between the effects (blood flow without GSK2256294 and blood flow with GSK2256294, both under the

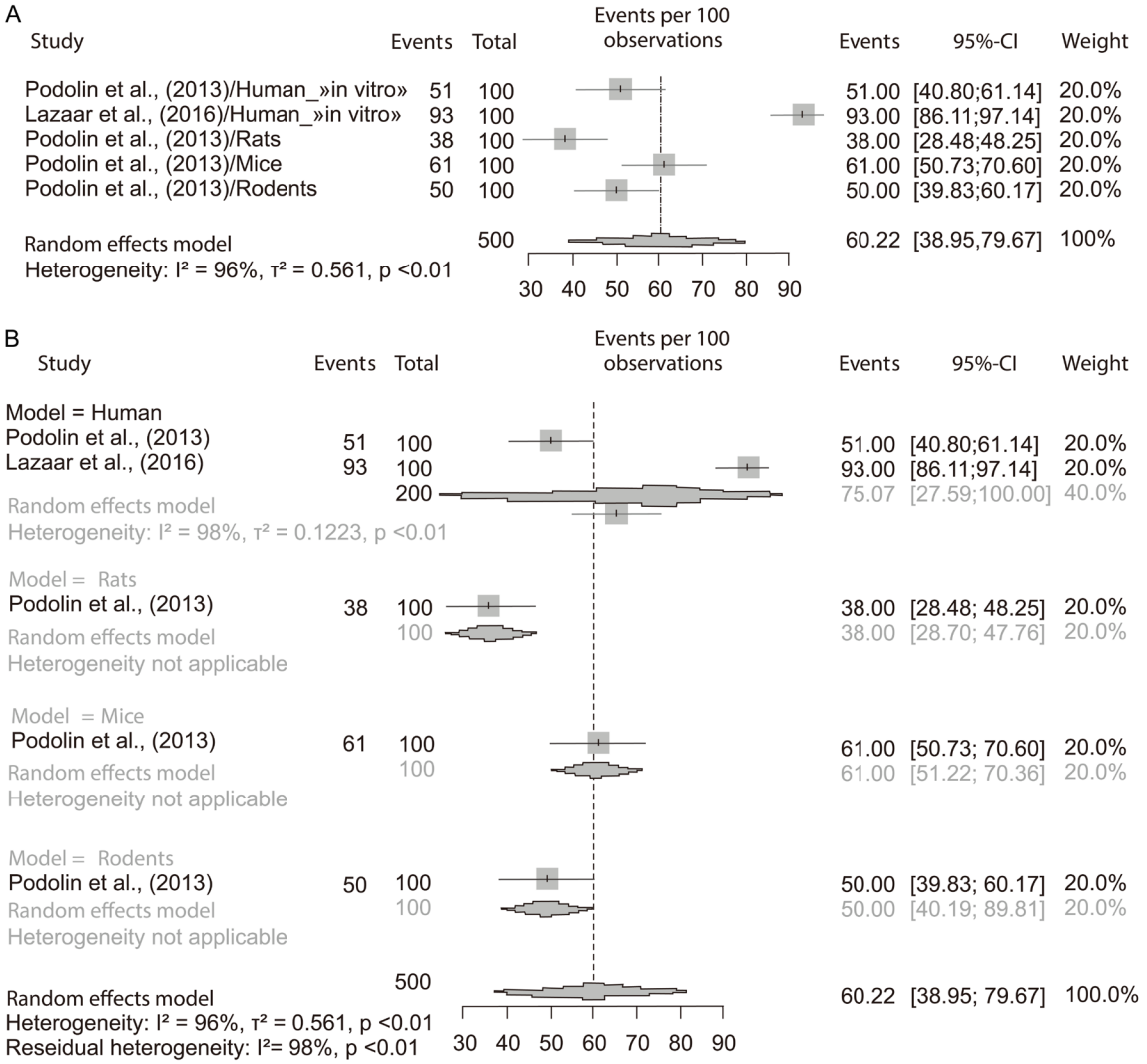

Figure 2. Evaluation of the effects of GSK2256294 on soluble epoxide hydrolase inhibition in a clinical and preclinical study of lung injury. A. Relative inhibition frequencies for the different study models. B. Relative frequencies of the inhibition effects stratified by study models. Prevalence means were used, and 95% confidence intervals were presented for each description.

effect of bradykinin) were analyzed. On average, blood flow increased by 23% (CI = 1.01 to 1.45) in individuals administered GSK2256294 compared with those who were not administered GSK2256294 (**Figure 5**).

Discussion

EETs, which are products of arachidonic acid epoxidation by cytochrome P450 enzymes, exert anti-inflammatory effects. EETs are metabolized by cyclooxygenase (COX) and β-oxidation but are mainly converted into diols by sEH. Pharmacological inhibition of sEH inhibits inflammation via a decrease in endothelial cell adhesion molecule expression induced by cytokines and chemokines and prevents leukocyte adhesion to vascular walls and tissues through a mechanism involving NF-κB inhibition [34, 35].

EETs can inhibit NF-κB activation, preventing its translocation to the cell nucleus, where it normally activates the transcription of inflammatory genes. This effect is likely mediated by specific receptors to which EETs bind and modulate intracellular signaling [36]. EETs can interfere with the activation of genes that encode pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1, thereby reducing the production of

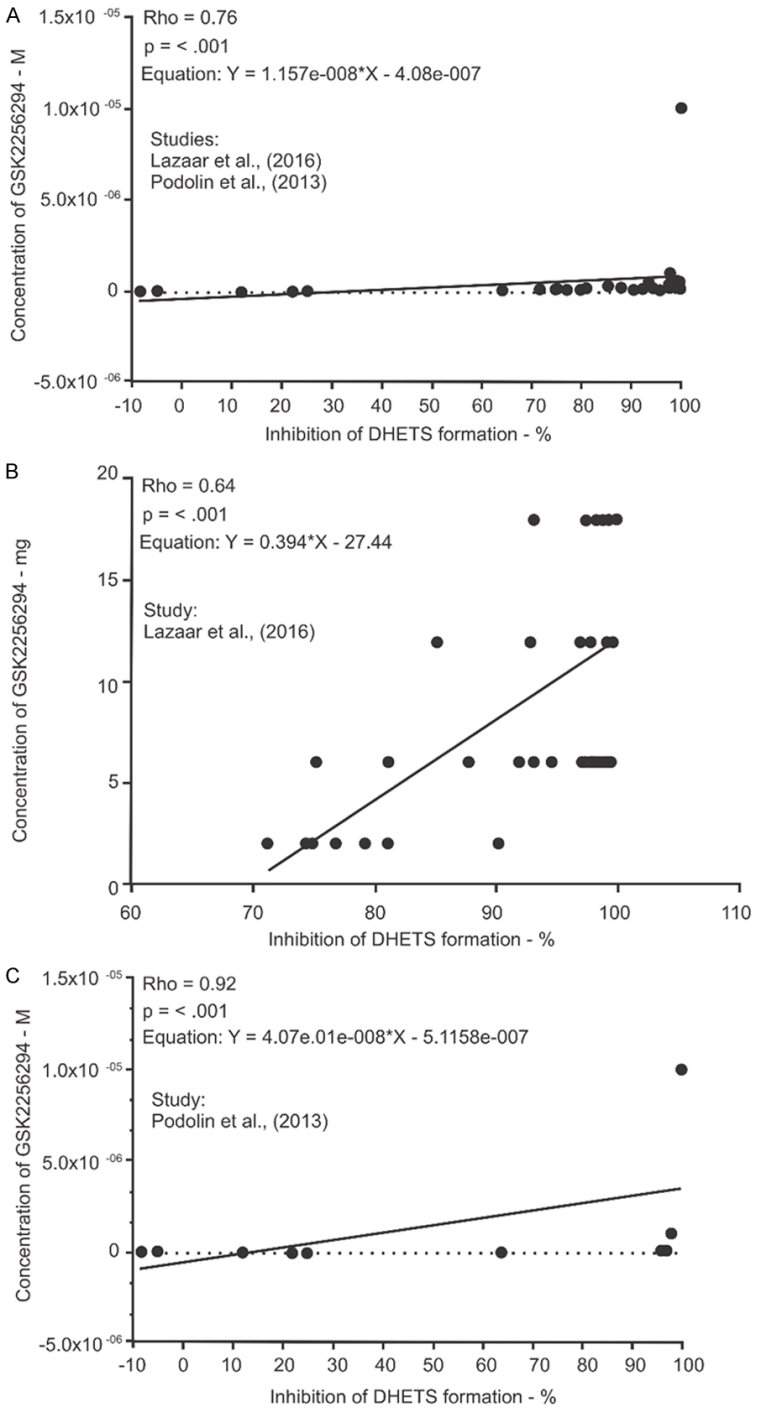

Figure 3. Correlation and meta-regression analyses of GSK2256294 concentration and DHETS formation inhibition in the eligible studies. A. Correlation between the effect and concentration of GSK2256294 and DHETS formation inhibition in preclinical and clinical models. B, C. Evaluations were conducted separately using each study model. Spearman's test was used to assess possible correlations. The significance level was 5%.

these cytokines [37, 38]. Such reductions can be achieved by modulating the activities of specific transcription factors or epigenetic processes. Moreover, modulation of ion channels, such as calcium and potassium channels, is another proposed mechanism [39].

Wang et al. [35] used experimental models to compare the effectiveness of different types of soluble epoxide hydrolase inhibitors (sEHIs), such as the acid sEHI t-TUCB (trans-4-{4-[3-(4-trifluoromethoxyphenyl)-ureido]-cyclohexyloxy}-benzoic acid) and a phosphodiesterase-4 (PDE4) inhibitor, at reducing lung injury and inflammation following subacute exposure to tobacco smoke. The sEH to diol ratio significantly decreased in the experimental animals, implying sEH activity.

Lung tissues and cells that express high sEH levels are considered as more susceptible to leukotoxins than those expressing low enzyme levels, thereby increasing their susceptibility to leukotoxin-diol, which is responsible for pulmonary toxicity [40]. Based on data collected from clinical [22] and preclinical [23] studies using GSK2256294, the conversion of leukotoxin to leukotoxin diol decreased in both experimental and human models.

Many clinical and preclinical studies have reported promising discoveries regarding pulmonary inflammation treatment. However, further data are needed to explain the current findings, which include significant differences between the results of sEH inhibition *in vitro* and *in vivo* [22, 23]. Capozzi et al. [41] revealed different outcomes regarding the effects of sEH inhibition, with sig-

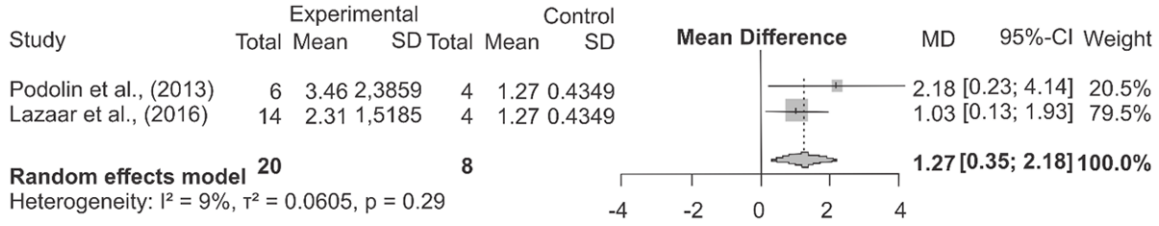

Figure 4. Mean distribution of the leukotoxin-to-leukotoxin-diol ratio in preclinical and clinical pulmonary inflammation models after intervention with GSK2256294. Sampling, means, and standard deviations for the effects on the leukotoxin-to-leukotoxin-diol ratio were obtained from the eligible and pooled studies. The randomized effect model was used. The significance level was 5%.

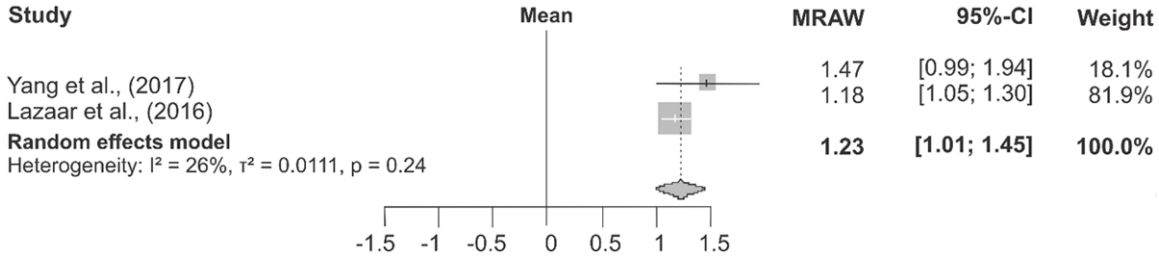

Figure 5. Effect of GSK2256294 on blood flow associated with bradykinin in a clinical study of lung injury in smokers. The mean effect of GSK2256294 was determined after the mean ratio was calculated between the control group (without GSK2256294 with 300 mg of bradykinin) and group treated with GSK2256294 (plus 300 mg of bradykinin). Summarization was performed using a randomized design, and confidence intervals were determined. The significance level was 5%.

nificantly better *in vivo* than *in vitro* results. Preclinical and clinical studies can be compared, as demonstrated by An et al. [42] Although the use of an sEHI was found to be safe in phase 1 of the trial, an appropriate efficacy level was not found in the phase 2 trial, as the beneficial effects found in animals were not replicated. The opposite result was obtained with GSK2256294, which, despite a negligible difference between interspecies results, produced positive outcomes in humans.

Treating COPD with anti-inflammatory compounds remains challenging because inflammation and related comorbidities are complex. Currently, no therapy effectively reverses the disease; therefore, minimizing disease progression is an alternative strategy, and reducing oxidative stress and inflammation can improve the quality of life and increase survival [43]. Oxidative stress, particularly that generated by exposure to tobacco smoke, is a major contributor to the pathogenesis of COPD, inducing apoptosis, extracellular matrix remodeling, protease inhibitor inactivation, mucus secretion, NF-κB activation, mitogen-activated protein kinase (MAPK) activation, chromatin remodeling, and pro-inflammatory gene transcription [43].

Arachidonic acid cascade modulators have been the focus of research in inflammation treatment for decades. Besides the known dual 5-lipoxygenase and cyclooxygenase-2 inhibitors, recent findings shed light on multi-target inhibitors that interfere with the cytochrome P450 pathway by inhibiting sEH, which offers a new opportunity for the development of novel anti-inflammatory drugs [44, 45].

Clinical studies using sEH inhibitors are limited. The selective sEH inhibitor AR9281 (Arete Pharmaceuticals) improved endothelial function in animal models, inhibited enzyme activity, and caused a modest decrease in dihydroxylipid levels in a clinical study involving healthy participants. A phase 2 study evaluating the effects of this inhibitor on blood pressure and glucose metabolism in patients with moderate hypertension and impaired glucose tolerance was terminated (NCT00847899). Other sEHIs have been tested in animal models of inflam-

matory diseases and have been demonstrated to increase EET/EDP levels and mitigate inflammation [16, 44]. Besides GSK2256294, other sEH inhibitors have been evaluated in experimental models of inflammatory lung injury. Notably, an increase in EET levels could be detected, consequently resulting in a reduction in DHETs and inflammation attenuation [46]. Given the selectivity and stability of some sEH inhibitors, their biological activities may vary in a model-dependent manner, resulting in the ineligibility of some inhibitors for clinical trials.

GSK2256294 ((1R, 3S)-N-(4-cyano-2-(trifluoromethyl)benzyl)-3-((4-methyl-6-(methylamino)-1,3,5-triazin-2-yl)amino)cyclohexanecarboxamide) is a potent inhibitor of strong but reversible binding of sEH. GSK2256294 belongs to the amide family and interacts with the amino acid residue, Asp333, of the catalytic triad, Asp333-Asp495-His523, which is responsible for opening the epoxide ring, and two tyrosine residues, Tyr381 and Tyr465, which are responsible for fixing the epoxide oxygen atom. Therefore, GSK2256294 is a competitive inhibitor that decreases the transcription of pro-inflammatory vascular cell adhesion molecule-1 (VCAM-1) and nuclear translocation of NF-κB in the presence of sHE in TNFα-activated human endothelial cells, thereby blocking the adhesion of mononuclear cells to the endothelium [17]. This inhibitor is specific to the hydrolase domain of EPHX2, inactive against the phosphatase domain, and attenuates cigarette smoke-induced lung inflammation in animal models.

Therefore, GSK2256294 can bind to the enzymatic catalytic site, inactivating its function, thereby increasing EET levels and decreasing the conversion to DHETs and leukotoxin-diol [15].

sEH hydrolyzes fatty acid epoxides to form diols, DHETs, and leukotoxin diols, with different biological functions and activities under various physiological and pathological conditions [47].

EETs can act as anti-inflammatory agents owing to several properties they exhibit in response to inflammatory processes. The elevation of EETs due to GSK2256294 use is related to its vasodilatory properties, which relaxes blood vessels and promotes increased blood flow [19, 48]. This can help reduce inflammation because vasodilation increases oxygen and nutrient delivery to inflamed tissues, contributing to the resolution of the inflammatory process. EETs inhibit platelet aggregation and reduce blood clot formation. This is important for preventing the inflammation associated with thrombotic disorders [49]. EETs can act on endothelial cells lining blood vessels, promoting healthy endothelial function and reducing the adhesion of leukocytes (immune cells) to blood vessels, which is an important step in the inflammatory response [50]. However, increasing epoxide levels and reducing inflammation have been proposed as rational therapeutic methods.

Some studies also revealed a possible decrease in cytokine expression, with modulation of pathways that converge in NF-κB-dependent transcription [31, 41]. These findings corroborate the therapeutic potential of this drug in patients with COPD.

One limitation of this study is the small number of primary studies included in this research. In addition, low consistency among the results of eligible studies affected the level of evidence reported in this secondary study.

Conclusions

GSK2256294 was found to reduce sEH enzyme levels in clinical and preclinical models, with greater effectiveness observed in clinical than preclinical studies. GSK2256294 also contributes to the anti-inflammatory activity mediated by the eicosatrienoic pathway by reducing DHETS and leukotoxin-diol and helping control blood flow. Thus, this study revealed that GSK2256294 is a promising drug for controlling the deleterious manifestations of pulmonary inflammation. However, given the low number of articles retrieved from the databases, more studies are required to guarantee the consistency of the evidence and unveil other pathways of biological activities mediated by GSK2256294.

Acknowledgements

This work was supported by the Federal University of Triângulo Mineiro (UFTM), University Center of Mineiros (UNIFIMES), and author W.R. received a Junior Postdoctoral Fellowship (PDJ) from the National Council for Scientific

and Technological Development (CNPq) (process: 152889/2022-1).

Disclosure of conflict of interest

None.

Address correspondence to: Wellington F Rodrigues, Federal University of Triângulo Mineiro, No. 30 Frei Paulino Street, Uberaba, MG 38025-180, Brazil. Tel: +55-034-99630-9185; E-mail: pos-doc.wellingtonrodrigues@uftm.edu.br

References

[1] Monserrat-Mesquida M, Quetglas-Llabrés M, Capó X, Bouzas C, Mateos D, Pons A, Tur JA and Sureda A. Metabolic syndrome is associated with oxidative stress and proinflammatory state. Antioxidants (Basel) 2020; 9: 236.

[2] Yang L, Cheriyan J, Gutterman DD, Mayer RJ, Ament Z, Griffin JL, Lazaar AL, Newby DE, Tal-Singer R and Wilkinson IB. Mechanisms of vascular dysfunction in COPD and effects of a novel soluble epoxide hydrolase inhibitor in smokers. Chest 2017; 151: 555-563.

[3] Salek-Ardakani S, So T, Halteman BS, Altman A and Croft M. Differential regulation of Th2 and Th1 lung inflammatory responses by protein kinase C theta. J Immunol 2004; 173: 6440-6447.

[4] Segal LN, Clemente JC, Tsay JC, Koralov SB, Keller BC, Wu BG, Li Y, Shen N, Ghedin E, Morris A, Diaz P, Huang L, Wikoff WR, Ubeda C, Artacho A, Rom WN, Sterman DH, Collman RG, Blaser MJ and Weiden MD. Enrichment of the lung microbiome with oral taxa is associated with lung inflammation of a Th17 phenotype. Nat Microbiol 2016; 1: 16031.

[5] de Araújo EF, Preite NW, Veldhoen M, Loures FV and Calich VLG. Pulmonary paracoccidioidomycosis in AhR deficient hosts is severe and associated with defective Treg and Th22 responses. Sci Rep 2020; 10: 11312.

[6] Lino-dos-Santos-Franco A, Correa-Costa M, Durão AC, de Oliveira AP, Breithaupt-Faloppa AC, Bertoni Jde A, Oliveira-Filho RM, Câmara NO, Marcourakis T and Tavares-de-Lima W. Formaldehyde induces lung inflammation by an oxidant and antioxidant enzymes mediated mechanism in the lung tissue. Toxicol Lett 2011; 207: 278-285.

[7] van der Vliet A. NADPH oxidases in lung biology and pathology: host defense enzymes, and more. Free Radic Biol Med 2008; 44: 938-955.

[8] Theodorakopoulou MP, Alexandrou ME, Bakaloudi DR, Pitsiou G, Stanopoulos I, Kontakiotis T and Boutou AK. Endothelial dysfunction in COPD: a systematic review and meta-analysis of studies using different functional assessment methods. ERJ Open Res 2021; 7: 00983-02020.

[9] Campbell WB and Falck JR. Arachidonic acid metabolites as endothelium-derived hyperpolarizing factors. Hypertension 2007; 49: 590-596.

[10] Krüger-Genge A, Blocki A, Franke RP and Jung F. Vascular endothelial cell biology: an update. Int J Mol Sci 2019; 20: 4411.

[11] Morisseau C and Hammock BD. Impact of soluble epoxide hydrolase and epoxyeicosanoids on human health. Annu Rev Pharmacol Toxicol 2013; 53: 37-58.

[12] Spector AA and Norris AW. Action of epoxyeicosatrienoic acids on cellular function. Am J Physiol Cell Physiol 2007; 292: C996-C1012.

[13] Ding Y, Belyanskaya S, DeLorey JL, Messer JA, Joseph Franklin G, Centrella PA, Morgan BA, Clark MA, Skinner SR, Dodson JW, Li P, Marino JP Jr and Israel DI. Discovery of soluble epoxide hydrolase inhibitors through DNA-encoded library technology (ELT). Bioorg Med Chem 2021; 41: 116216.

[14] Titz B, Luettich K, Leroy P, Boue S, Vuillaume G, Vihervaara T, Ekroos K, Martin F, Peitsch MC and Hoeng J. Alterations in serum polyunsaturated fatty acids and eicosanoids in patients with mild to moderate chronic obstructive pulmonary disease (COPD). Int J Mol Sci 2016; 17: 1583.

[15] Dong XW, Jia YL, Ge LT, Jiang B, Jiang JX, Shen J, Jin YC, Guan Y, Sun Y and Xie QM. Soluble epoxide hydrolase inhibitor AUDA decreases bleomycin-induced pulmonary toxicity in mice by inhibiting the p38/Smad3 pathways. Toxicology 2017; 389: 31-41.

[16] Chung KF. Airway smooth muscle cells: contributing to and regulating airway mucosal inflammation? Eur Respir J 2000; 15: 961-968.

[17] Belyanskaya SL, Ding Y, Callahan JF, Lazaar AL and Israel DI. Discovering drugs with DNA-encoded library technology: from concept to clinic with an inhibitor of soluble epoxide hydrolase. Chembiochem 2017; 18: 837-842.

[18] Mashayekhi M, Wanjalla CN, Warren CM, Simmons JD, Ghoshal K, Pilkinton M, Bailin SS, Gabriel CL, Pozzi A, Koethe JR, Brown NJ, Kalams SA and Luther JM. The soluble epoxide hydrolase inhibitor GSK2256294 decreases the proportion of adipose pro-inflammatory T cells. Prostaglandins Other Lipid Mediat 2022; 158: 106604.

[19] Luther JM, Ray J, Wei D, Koethe JR, Hannah L, DeMatteo A, Manning R, Terker AS, Peng D, Nian H, Yu C, Mashayekhi M, Gamboa J and Brown NJ. GSK2256294 decreases sEH (soluble epoxide hydrolase) activity in plasma, mus-

cle, and adipose and reduces F2-isoprostanes but does not alter insulin sensitivity in humans. Hypertension 2021; 78: 1092-1102.
[20] Dai M, Wu L, He Z, Zhang S, Chen C, Xu X, Wang P, Gruzdev A, Zeldin DC and Wang DW. Epoxyeicosatrienoic acids regulate macrophage polarization and prevent LPS-induced cardiac dysfunction. J Cell Physiol 2015; 230: 2108-2119.
[21] Zhang J, Zhang M, Huo XK, Ning J, Yu ZL, Morisseau C, Sun CP, Hammock BD and Ma XC. Macrophage inactivation by small molecule wedelolactone via targeting sEH for the treatment of LPS-induced acute lung injury. ACS Cent Sci 2023; 9: 440-456.
[22] Lazaar AL, Yang L, Boardley RL, Goyal NS, Robertson J, Baldwin SJ, Newby DE, Wilkinson IB, Tal-Singer R, Mayer RJ and Cheriyan J. Pharmacokinetics, pharmacodynamics and adverse event profile of GSK2256294, a novel soluble epoxide hydrolase inhibitor. Br J Clin Pharmacol 2016; 81: 971-979.
[23] Podolin PL, Bolognese BJ, Foley JF, Long E 3rd, Peck B, Umbrecht S, Zhang X, Zhu P, Schwartz B, Xie W, Quinn C, Qi H, Sweitzer S, Chen S, Galop M, Ding Y, Belyanskaya SL, Israel DI, Morgan BA, Behm DJ, Marino JP Jr, Kurali E, Barnette MS, Mayer RJ, Booth-Genthe CL and Callahan JF. In vitro and in vivo characterization of a novel soluble epoxide hydrolase inhibitor. Prostaglandins Other Lipid Mediat 2013; 104-105: 25-31.
[24] Sencar ME, Calapkulu M, Sakiz D, Hepsen S, Kus A, Akhanli P, Unsal IO, Kizilgul M, Ucan B, Ozbek M and Cakal E. An evaluation of the results of the steroid and non-steroidal anti-inflammatory drug treatments in subacute thyroiditis in relation to persistent hypothyroidism and recurrence. Sci Rep 2019; 9: 16899.
[25] Bednarek D, Szuster-Ciesielska A, Zdzisińska B, Kondracki M, Paduch R and Kandefer-Szerszeñ M. The effect of steroidal and non-steroidal anti-inflammatory drugs on the cellular immunity of calves with experimentally-induced local lung inflammation. Vet Immunol Immunopathol 1999; 71: 1-15.
[26] Iyer A, Kauter K, Alam MA, Hwang SH, Morisseau C, Hammock BD and Brown L. Pharmacological inhibition of soluble epoxide hydrolase ameliorates diet-induced metabolic syndrome in rats. Exp Diabetes Res 2012; 2012: 758614.
[27] Page MJ, Moher D, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P and McKenzie JE. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. BMJ 2021; 372: n160.
[28] Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, Cates CJ, Cheng HY, Corbett MS, Eldridge SM, Emberson JR, Hernán MA, Hopewell S, Hróbjartsson A, Junqueira DR, Jüni P, Kirkham JJ, Lasserson T, Li T, McAleenan A, Reeves BC, Shepperd S, Shrier I, Stewart LA, Tilling K, White IR, Whiting PF and Higgins JPT. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ 2019; 366: l4898.
[29] Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M and Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol 2014; 14: 43.
[30] Arango HG. Bioestatística teórica e computacional. In: Bioestatística Teórica e Computacional Rio de Janeiro, editora. Guanabara Koogan; 2001. pp. 235-235.
[31] Higgins JP and Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002; 21: 1539-1558.
[32] Littell JH, Corcoran J and Pillai V. Systematic reviews and meta-analysis. New York: Oxford University Press; 2008. pp. 1-76.
[33] Viechtbauer W. Conducting meta-analyses in R with the metafor package. J Stat Softw 2010; 36: 1-48.
[34] Norwood S, Liao J, Hammock BD and Yang GY. Epoxyeicosatrienoic acids and soluble epoxide hydrolase: potential therapeutic targets for inflammation and its induced carcinogenesis. Am J Transl Res 2010; 2: 447-457.
[35] Wang T, Palucci D, Law K, Yanagawa B, Yan J and Butany J. Atherosclerosis: pathogenesis and pathology. Diagn Histopath 2012; 18: 461-467.
[36] Das Mahapatra A, Choubey R and Datta B. Small molecule soluble epoxide hydrolase inhibitors in multitarget and combination therapies for inflammation and cancer. Molecules 2020; 25: 5488.
[37] Hoxha M and Zappacosta B. CYP-derived eicosanoids: implications for rheumatoid arthritis. Prostaglandins Other Lipid Mediat 2020; 146: 106405.
[38] Trindade-da-Silva CA, Clemente-Napimoga JT, Abdalla HB, Rosa SM, Ueira-Vieira C, Morisseau C, Verri WA Jr, Montalli VAM, Hammock BD and Napimoga MH. Soluble epoxide hydrolase inhibitor, TPPU, increases regulatory T cells pathway in an arthritis model. FASEB J 2020; 34: 9074.
[39] Bartra C, Irisarri A, Villoslada A, Corpas R, Aguirre S, García-Lara E, Suñol C, Pallàs M, Griñán-Ferré C and Sanfeliu C. Neuroprotective epigenetic changes induced by maternal treatment with an inhibitor of soluble epoxide hy-

drolase prevents early Alzheimer's disease neurodegeneration. Int J Mol Sci 2022; 23: 15151.
[40] Zheng J, Plopper CG, Lakritz J, Storms DH and Hammock BD. Leukotoxin-diol: a putative toxic mediator involved in acute respiratory distress syndrome. Am J Respir Cell Mol Biol 2001; 25: 434-438.
[41] Capozzi ME, Hammer SS, McCollum GW and Penn JS. Epoxygenated fatty acids inhibit retinal vascular inflammation. Sci Rep 2016; 6: 39211.
[42] An G, Lee KSS, Yang J and Hammock BD. Target-mediated drug disposition-a class effect of soluble epoxide hydrolase inhibitors. J Clin Pharmacol 2021; 61: 531-537.
[43] Rodrigues SO, Cunha CMCD, Soares GMV, Silva PL, Silva AR and Gonçalves-de-Albuquerque CF. Mechanisms, pathophysiology and currently proposed treatments of chronic obstructive pulmonary disease. Pharmaceuticals (Basel) 2021; 14: 979.
[44] Meirer K, Steinhilber D and Proschak E. Inhibitors of the arachidonic acid cascade: interfering with multiple pathways. Basic Clin Pharmacol Toxicol 2014; 114: 83-91.
[45] Ontko CD, Capozzi ME, Kim MJ, McCollum GW and Penn JS. Cytochrome P450-epoxygenated fatty acids inhibit Müller glial inflammation. Sci Rep 2021; 11: 9677.
[46] Tao W, Xu G, Luo Y and Li PS. Inhibitors of soluble epoxide hydrolase on acute lung injury: a meta-analysis of preclinical studies. Inflammopharmacology 2022; 30: 2027-2033.
[47] McReynolds C, Hammock B and Morisseau C. Regulatory lipid vicinal diols counteract the biological activity of epoxy fatty acids and can act as biomarkers and mechanisms for disease progression. Pharmacol Ther 2023; 248: 108454.
[48] Martini RP, Siler D, Cetas J, Alkayed NJ, Allen E and Treggiari MM. A double-blind, randomized, placebo-controlled trial of soluble epoxide hydrolase inhibition in patients with aneurysmal subarachnoid hemorrhage. Neurocrit Care 2022; 36: 905-915.
[49] Luo L, Yang Y, Fu M, Luo J, Li W, Tu L and Dong R. 11,12-EET suppressed LPS induced TF expression and thrombus formation by accelerating mRNA degradation rate via strengthening PI3K-Akt signaling pathway and inhibiting p38-TTP pathway. Prostaglandins Other Lipid Mediat 2023; 167: 106740.
[50] Kotlyarov S. Immune function of endothelial cells: evolutionary aspects, molecular biology and role in atherogenesis. Int J Mol Sci 2022; 23: 9770.

Supplementary Table 1. Assessment of the methodological quality of eligible clinical trials (RobBis 2)

Categories	Study - Randomized crossover	
	Aili L Lazaar, 2016	Lucy Yang, 2017
Domain 1	Some concerns	Low risk
Domain 2	Some concerns	Low risk
Domain 3	Low risk	Some concerns
Domain 4	Low risk	Low risk
Domain 5	Low risk	Low risk
Classification	Some concerns	Some concerns

Supplementary Table 2. Assessment of the methodological quality of eligible preclinical studies (SYRCLE)

Categories	Study - preclinical
	Patricia L Podolin, 2013
Domain 1	1
Domain 2	0.5; 0.5 and 1
Domain 3	0
Domain 4	1 and 1
Domain 5	0.5
Domain 6	0
Domain 7	0 and 0
Domain 8	1; 0.5; 1 and 1
Domain 9	0.5 and 1
Domain 10	1; 0.5; 1; 0 and 1
Classification	Moderate risk of bias

Classification: 0, high risk of bias; 0.5, moderate risk of bias; 1, low risk of bias.