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Molecular Biology of Chronic Obstructive Pulmonary Disease from the Bases to the Therapeutic Decision: A Review

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Authors' contributions

This work was carried out in collaboration between both authors. Authors AA-G and IA-J contributed equally in the planning, data collection, data analysis, writing and critical review. Both authors read and approved the final manuscript.

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a global public health problem. It has an overwhelming prevalence, yet accepted therapies are ineffective in reducing disease progression. Bronchodilators, the mainstay of COPD treatment, only provide symptomatic relief. Therefore, in order to provide a superior approach, it is important to better understand the rationale behind therapy and the underlying mechanisms by which the inflammatory process, through various pathogenic pathways, leads to deterioration. Cigarette smoke and other pollutants/biomass fuels affect the lungs ability to counterbalance proteases and neutralize different types of stress. Even if the initial noxa is discontinued, inflammation, infection and autoimmunity promote a chronic lung inflammatory response; leading to the development of emphysema and small airway disease. This is due to continuous endogenous production of reactive oxygen species, nitrative and carbonyl stress. The process then continues into a harmful spiral and systemic disease. The objective of this paper is to offer an updated review of COPD, simplifying the integration of basic science research and introducing the concepts and evidence of therapeutic alternatives. This review discusses why

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some drugs have failed and which alternatives are emerging. Probably there is no unique effective therapy, but several combinations of drugs might be required to impact the different subcellular compartments and obtain a more effective therapy in COPD.

Keywords: Chronic obstructive pulmonary disease; lung inflammation; oxidative stress; autoimmunity in COPD; molecular biology.

1. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a global public health problem. It is projected that by 2020 it will be the third leading cause of dead worldwide, and the fifth leading cause of years of life lost due to disability coupled with years of life lost due to premature dead (DALYs) [1]. The condition affects 10% of the world population above 45 years, and not only 15% of the total smokers but up to 50% of heavy smokers [2,3].

The definition provides that, besides being preventable, treatable and characterized by a chronic and persistent airflow limitation (usually progressive), COPD is due to an increased chronic inflammatory response [4]. The pathologic process is initiated by exposure to cigarette smoke or biomass fuels [5]. Exacerbations and many comorbidities that contribute to severity in a particular patient, have an inflammatory nature as well [6]. The inflammatory profile in normal smokers is very similar to that of patients with COPD, but less prominent. The concept that emerges is that of an amplified inflammatory response in COPD patients. It is important to understand how the inflammatory response at different anatomical sites causes different physiological sequels, pathological events and clinical manifestations: Central airways (chronic bronchitis), small peripheral airways (obstructive bronchiolitis), lung parenchyma (emphysema), cardiovascular system (pulmonary vascular disease and cor pulmonale) and dysfunction of respiratory and peripheral muscles (systemic disease). The inflammatory process in these compartments is similar, but COPD predominantly affects the small airways and the lung parenchyma [7,8].

The aim of this review is to dissect the molecular biology of the inflammatory process and discuss innovative therapeutic strategies for COPD.

2. INFLAMMATORY CELLS

Each inhalation of cigarette smoke contains about 10^{17} reactive oxygen species (ROS), which initiate the inflammatory response in airways and lung parenchyma. The amplified inflammatory response in COPD is associated with mucus production, proteolysis, fibrosis and cycles of resolution. The process is possibly determined by genetic factors [9-11], latent viruses [12,13], oxidative stress [14] and alteration of the Histone Deacetylase-2 (HDAC-2) activity [15]. The type of inflammation that occurs is mediated by the recruitment of different inflammatory cells and the production of distinct mediators, the most important ones will be discussed (Fig. 1).

Macrophages are increased in number and activity in the sputum and bronchoalveolar lavage (BAL) of patients with COPD; macrophages play a main role in the inflammatory process. Stimulated by cigarette smoke and other irritants, they release ROS, nitric oxide (NO) and chemokines that attract monocytes, neutrophils and T cells into the inflamed area [16]. Macrophages also have a longer life span, mediated by increased activity of Bc1 -XL anti apoptotic protein [17].

T cells are CD8 + (suppressor/cytotoxic) subtype Th1/Tc1 (producers of γ-interferon) and are located in mucus secreting glands, central and peripheral airways and lung parenchyma. They release granzymes, perforins and tumor necrosis factor α (TNF-α), which induces apoptosis of alveolar type I cells, favoring emphysema [7,15].

Neutrophils are increased in the sputum and BAL of patients with COPD. They are attracted by epithelial cells, macrophages and T cells through chemotactic factors; such as interleukin 8 (IL-8), leukotriene B4 (LTB4) and a number of chemokines that belong to the CXC family (cytokines that act over R specific receptors). Neutrophils release serine proteases like Elastase, Cathepsin G, Proteinase-3, Matrix Metalloproteinase-12 (MMP-12) and toxic oxygen radicals, which promote the production of mucus and alveolar destruction [18].

Is controversial whether eosinophils are elevated in the sputum of stable COPD patients, although they are increased during exacerbation [19]. 10%

of COPD patients respond to inhaled glucocorticoids, these patients have a greater number of eosinophils in the airways and greater reversibility to bronchodilators. It has been suggested that this patients may have concomitant asthma [8]. Recently, in a joint effort of GINA and GOLD the term ACOS was developed, as an overlap syndrome between Asthma and COPD [20].

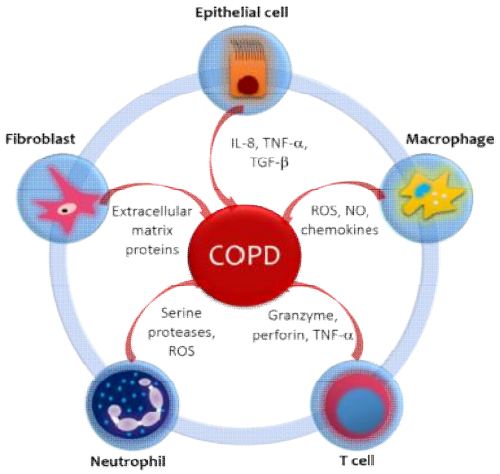

Fig. 1. Inflammatory and immune cells and mediators involved in COPD

Cigarette smoke and other pollutants/biomass fuels are the initial noxa that stimulate epithelial cells and macrophages to release chemotactic factors that attract other inflammatory cells to the lungs. The distinct pattern of inflammation in COPD determines the structural consequences and response to therapy. The circular disposition is a simplified model, but it aims to emphasize the confluence and interplay between the related cells and mediators. IL-8: Interleukin-8. TNF-α: Tissue Necrosis Factor-α. TGF-β Transforming Growth Factor β. ROS: Reactive Oxygen Species. NO: Nitric Oxide

Epithelial cells produce TNF-α and IL-8 in response to inhaled bronchial irritants. They also generate transforming growth factor β (TGF-β) which can cause local fibrosis. Fibroblasts have increased activity and produce extracellular matrix proteins in the small airways (obstructive bronchiolitis). Smooth muscle cells and endothelial cells are also involved in the inflammatory process [7].

3. INFLAMMATORY MEDIATORS

Many of the cytokines and chemokines that are secreted in COPD are regulated by the transcription factor nuclear factor-Kβ (NF-Kβ) (Fig. 2). TNF-α is produced by macrophages, epithelial cells, neutrophils and fibroblasts [8]. TNF-α activates transcription of NF- Kβ. NF-Kβ in turn activates inflammatory genes (such as IL-8 genes) and proteases in macrophages and epithelial cells [21]. It seems to be an increase in TNF-α in the sputum of COPD patients, compared with healthy smokers and asthmatics.

Patients with COPD who lose weight and are cachectic, have elevated TNF-α levels; not only in sputum but also in peripheral blood, derived from monocytes. Cachexia occurs by inactivation of skeletal muscle proteins (via activation of NF-Kβ) [22]. In addition to TNF-α and its receptors (TNFr-55 and TNFr-75), IL-6, IL-8, fibrinogen and C-reactive protein are elevated in the systemic circulation, generating an inflammatory response at this level.

IL-8 and other chemokines are secreted by neutrophils, macrophages and epithelial airway cells. Interestingly, the IL-8 increased sputum and BAL levels, correlate with the number of neutrophils and the degree of airflow obstruction [23]. IL-8 activates signals through 2 receptors: (1) CXCR1, which is a low affinity receptor, but is mostly activated by IL-8 and (2) CXCR2 which is a high IL-8 affinity receptor, but could be activated by several chemokines. Activation of these two receptors generates chemotaxis and adhesion of neutrophils, as well as activation of toxic oxygen radicals, myeloperoxidase (MPO) and enzymes. CXCR1 is involved in the activation of neutrophils and CXCR2 is expressed on neutrophils and monocytes [7].

LTB4 is elevated in the sputum of COPD patients (derived from alveolar macrophages and neutrophils) and acts as a potent chemotactic factor for neutrophils. TGF-β1 favors the fibrotic process of small airways. Interleukin 13 (IL-13) can induce pulmonary emphysema via MMP and cathepsin [24]. IL-1B and IL-1 may also produce emphysema through neutrophil elastase. Their receptors could be therapeutic targets in this entity thru small molecules or antibodies [25]. Endothelin-1 (ET-1) is involved in pulmonary vascular remodeling in COPD and pulmonary associated hypertension [26,27].

4. PROTREASES-ANTIPROTEASES IMBALANCE

Lung parenchymal destruction (emphysema) is caused by an imbalance between proteases, that degrade the connective tissue of alveolar walls, and anti-proteases that protect against this

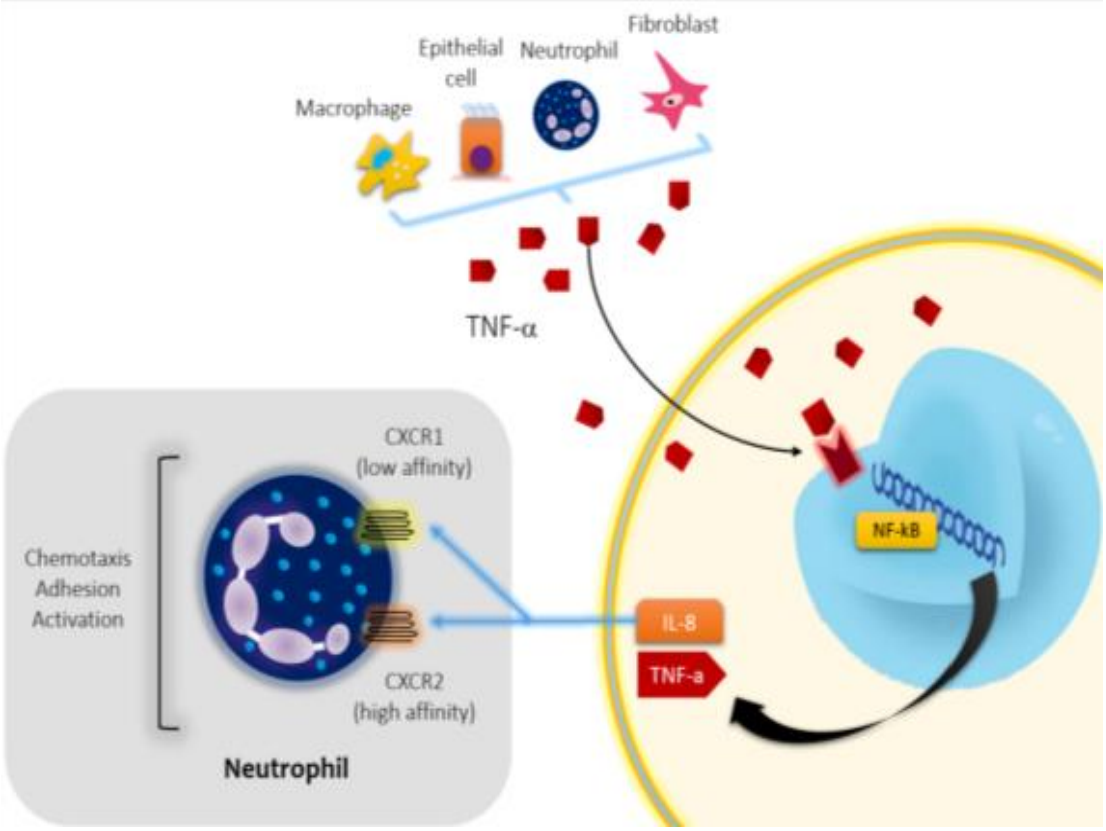

Fig. 2. Activation of the Nuclear Factor-Kβ (NF-Kβ)

NF-Kβ has a central role in orchestrating the inflammatory response in COPD. NF-Kβ is activated by oxidants and inflammatory mediators such as TNF-α produced by macrophage, epithelial cells, neutrophils and fibroblasts. Most of the inflammatory proteins that are upregulated in COPD macrophages are regulated by NF-Kβ. Interleukin-8 (IL-8) is a ubiquitous inflammatory chemokine that mediates several inflammatory events in the lungs, it is a major chemotactic and activating mediator of neutrophils. TNF-α.: Tissue Necrosis Factor-α. NF-Kβ Nuclear Factor- Kβ. CXCR1: chemokine C-X-C motif receptor 1. CXCR2: chemokine C-X-C motif receptor 2

damage [4]. Neutrophil elastase is a serine protease with potent elastolytic activity, it also induces IL-8 release from epithelial cells (perpetuating the inflammatory process). Proteinase-3 and cathepsin G are other neutrophil serine proteases that contribute to elastolysis. Cathepsins B, K, L and S are cysteine proteases, with similar elastolytic activity, but derived from macrophages.

Matrix Metalloproteinases (MMPs) are a group of endopeptidases that degrade the matrix of lung parenchyma, including elastin, collagen, laminin, fibronectin and proteoglycans. They are produced by neutrophils, macrophages and epithelial cells in response to IL-IB, TNF-α and ROS.

Opposite to these proteases are anti-proteases, such as α1- antitrypsin (α1-AT). α1-AT has the highest anti-protease activity in lung parenchyma, it is derived from plasma and is produced in the liver. α1-AT homozygous deficit produces severe emphysema (particularly in smokers), but accounts for less than 1% of COPD cases. Reduced α1-Antichymotripsine is also a risk factor for COPD.

Secretory leukocyte protease inhibitor (SLPI), comes from epithelial cells and is the primary protective anti-protease in airways. Tissue Inhibitor of Metalloproteinases (TIMPs) counteracts the effect of metalloproteinases. Oxidative stress potently inhibits α1-AT and SLPI and promotes the activation of MMPs, polarizing balance to degradation by proteases. Furthermore, MMP-9 activates TGF-β1, establishing a close relationship between proteolysis and fibrosis [28-30].

5. STRESS IN COPD

Oxidative stress is one of the most important predisposing mechanisms in the pathogenesis of COPD (Fig. 3). It occurs as a result of the increased exposure to ROS (exogenous and endogenous), and a further reduced antioxidant defense (overwhelmed or genetically altered) [31]. The most destructive oxidant molecules are H_2O_2 or hydrogen peroxide (acting as a substrate to generate additional oxidant molecules), ethane, isoprostanes (produced by direct oxidation of arachidonic acid), hydroxyl radical (-OH) and superoxide radical (O^{2-}). Oxidant molecules are elevated in the condensate of expired air of COPD patients, especially during exacerbations.

Exogenous oxidant sources include cigarette smoke, oxidizing gases, ultrafine particulate material and nanoparticles from industrial pollution, exhausted vapors and biomass fuels for cooking and heating homes (particularly in third world countries) [32,33]. Endogenous oxidants result mainly from mitochondrial respiration and inflammatory responses to viruses and bacteria. Inflammatory stress mediated by IL-1, TNF-α, and interferon-γ generates endogenous ROS. Other sources of intracellular ROS are NADPH oxidase enzyme, xanthine oxidase (XO) and hem peroxidases; all of which are increased in the BAL and in the airway inflammatory cells of COPD patients [34,35].

Free radicals inherent instability initiates an oxidative process, and a number of adverse consequences at a cellular level. For example, oxidants activate NF-kβ increasing the synthesis of IL-8 and TNF-α which recruit neutrophils and perpetuate the inflammatory stress. Oxidative stress also activates PI3K-δ (Phosphoinositide 3-Kinase-δ), which phosphorylates HDAC-2 (a key anti-inflammatory enzyme) and inactivates it, subsequently suffering ubiquitination and proteosomic degradation [36].

There is an interplay between pathological mechanisms, and oxidants also contribute to the protease/anti-protease imbalance, by reducing anti-protease activity (e.g. α1-AT and SLPI). This is the case of neutrophil-derived MPO, which produces the very destructive hypochlorous acid, and in turn it inactivates α1-AT. Furthermore, oxidants mediate plasma exudation, increase mucus secretion and smooth muscle contraction [7].

In non-pathological conditions, lungs constant exposure to exogenous and endogenous sources of oxidative stress has contributed to the development of potent antioxidant strategies. For instance, up to 20% of reduced glutathione (GSH) is located within the mitochondria, disposed to neutralize endogenous ROS (byproduct of metabolism). Also, the lung environment-exposed surface contains antioxidants, such as ascorbic acid (vitamin C), α-tocopherol (vitamin E) and bilirubin. Larger molecules, such albumin and mucin, have exposed sulfhydryl groups that act as antioxidants too. Several studies have described an association between impaired lung function in COPD and low levels of lung antioxidants [37].

TGF-β expression is increased in COPD, it inhibits the expression of the antioxidant enzymes catalase and superoxide dismutase 2 (SOD2). Both are critical to neutralize ROS derived from mitochondria and are under the control of the transcription factor FOXO3, whose deficiency has been associated with COPD [38]. Over 200 cellular antioxidant and detoxification enzymes are under the control of Nrf2 (nuclear factor-erythroid 2- related factor 2) [39]. COPD patients have reduced expression and activity of Nrf2. Upregulation or restoration of Nrf2 activity may, therefore, prove to be of therapeutic benefit in COPD [8].

Nitrative stress is due to the potent radical peroxynitrite ($ONOO^-$), which is formed by the reaction of superoxide anion (O_2^-) with nitric oxide (NO); at the same time it generates the reactive OH^-. The reaction of $ONOO^-$ with certain proteins and enzymes (i.e. nitration), reduces their activity and expression. For example, nitration of HDAC-2 enzyme inactive it [36]. Carbonyl stress occur when ROS oxidize proteins, lipids, carbohydrates and DNA; producing reactive carbonyls, which in turn react with proteins. This is known as protein carbonylation, a non-enzymatic phenomenon that occurs in specific amino acid residues, such as lysine, arginine, cysteine and histidine [40]. Carbonylation distorts protein function, normal cell function and physiological mechanisms [41], it occurs in both smokers and COPD patients [42]. Endoplasmic reticulum oxidative stress can induce mitochondrial apoptosis and cell death [43].

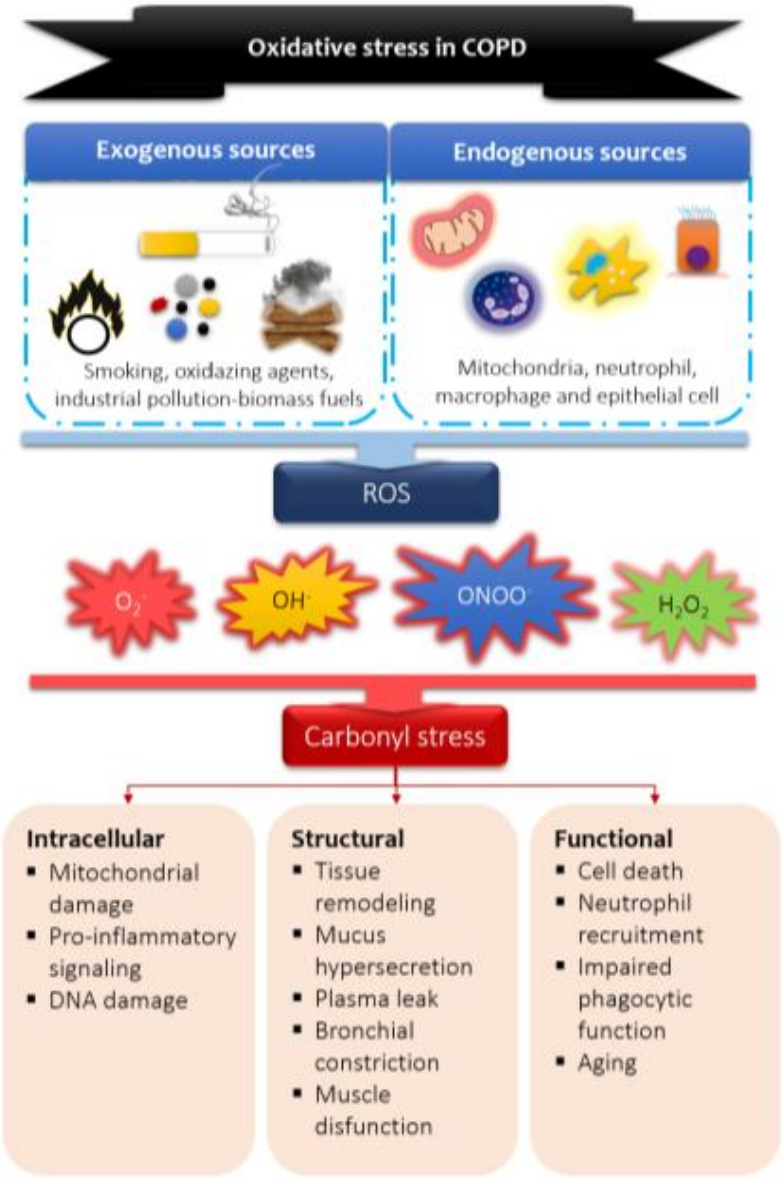

Fig. 3. Model of the role of oxidative stress in COPD

Environmental and endogenous sources of Reactive Oxygen Species (ROS) cause lipid peroxidation and the oxidation of proteins and carbohydrates, leading to carbonyl stress. Carbonyl stress in turn produces detrimental intracellular, structural and functional processes. O^{2-}: superoxide radical. -OH: hydroxyl radical. ONOO$^-$: peroxynitrite. H_2O_2: hydrogen peroxide

6. AUTOIMMUNITY IN COPD

One essential characteristic of COPD is that the inflammatory process and stress continue after stopping exposure to irritants [44]. It is likely that persistent infection and autoimmunity are responsible for this behavior. Carbonyl-modified proteins are highly immunogenic, producing autoantibodies that are elevated in the serum of patients with COPD [45]. These autoantibodies bind the complement, and may also contribute to emphysema.

Carbonylated proteins are recognized by the innate immune system through the PRRs (Pattern Recognition Receptors); expressed in

cells that identify antigens, such as dendritic cells and macrophages [46]. In these cells, they are processed and re-expressed in association with the Major Histocompatibility Complex-2 (HLA-2). This promotes the activation of the acquired immune response and attracts and accumulates Th1 cells in the lung parenchyma and dendritic cells in small airways [47].

It is unclear whether this immune response is a protective or destructive epiphenomenon. It is likely to be detrimental, given that autoantibodies are of the IgG1 isotype (potentially destructive) and bind the component C3 of the complement [48]. Besides producing neo-antigens, the immune response also promotes the influx of the immune cells needed to recognize and process them. This stimulus causes the release of CCL2 and CCL20, which recruit dendritic cells, monocytes and lymphocytes. In order to improve the immune response, IL-17 and IL-18 levels are increased. This interleukins activate and mature B cells, and also promote autoimmunity [49].

7. INFECTION AND EXACERBATION IN COPD

While it is true that cigarette smoking is the major primary risk factor for COPD, respiratory infections may play a role in the development and progression of the disease; and are also the leading cause of acute exacerbations [50]. Tobacco smoke and infection lead to differential activation of multiple PRRs. When it comes to infection, these receptors are activated by PAMPs (Pathogen Associated Molecular Patterns) and DAMPs (Damage Associated Molecular Patterns). DAMPs are endogenous molecules released by lung tissue after injury, infectious or noninfectious [51].

There are at least 4 groups of clearly identified PRRs that receive and process these signals. Toll-Like Receptors (TLR), NOD-Like Receptors (NLR) and cytosolic DNA sensors, which sense bacterial PAMPs; and RIG-I-like Receptors (RIG-I), that sense viral PAMPs [52]. NLRP1-3 generates inflammasomes, which are aggregates of multiple protein complexes that mediate inflammation. As previously discussed, cells that express PRRs generate cytokines, interferons and chemokines that recruit macrophages, neutrophils and activate epithelial cells; the innate immune response. Dendritic cells, stimulated by PRRs ligands and associated to HLA-2, initiate signals that attract T cells; the adaptive immune response.

COPD patients have an increased colonization by *H. influenza*, *S. pneumoniae*, *P. aeruginosa* and *M. catarrhalis*, this contributes to chronic inflammation and airway dysfunction. Infection with these and other pathogens (including virus) is a major cause of acute exacerbations [50]. Increased susceptibility of COPD patients to these infections appear to be related to a dysfunctional innate immune system, along with an altered mucociliary clearance.

It is important to emphasize the PRRs role in COPD, since they mediate the autoimmune and infectious responses: the two events that have been involved in the persistence of the inflammatory process. Process that becomes autonomous and does not disappear, even after exposure to irritants ceases.

8. THERAPEUTICS

Existing pharmacological treatments do not reduce the progression of COPD. Bronchodilators (which are the pharmacological basis of therapeutics) only provide symptomatic relief [53]. Pharmacological and medical investigations have searched for alternatives that attempt to impact the inflammatory process, and the mechanisms that perpetuate it. Some recent options are discussed below (Fig. 4).

The circular design was made to emphasize that there is no proven unique effective therapy for COPD, but that several combinations might be required. As discussed in the text steroids + theophylline, ACE inhibitors, IL-Receptor blockers and sulfonylureas have a predominant anti-inflammatory effect. The other drugs act mainly by anti-oxidant mechanisms. Key words of the rationale behind each therapy are provided. HDAC: histone deacetylase. FEV1: Forced expiratory volume in 1 second. IL-R: Interleukin Receptor. NLRP3: NOD-like Receptor family, pyrin domain containing 3. ROS: Reactive Oxygen Species. Nrf2: Nuclear factor-erythroid 2-related factor 2.

8.1 Increase α1-antitrypsin Activity

Young patients with severe deficiency of α-1 AT and established emphysema may be candidates for α1-AT therapy (Evidence C). However, this therapy is very expensive, is not available in many countries and is not indicated for patients with COPD unrelated to α1-AT deficiency [4].

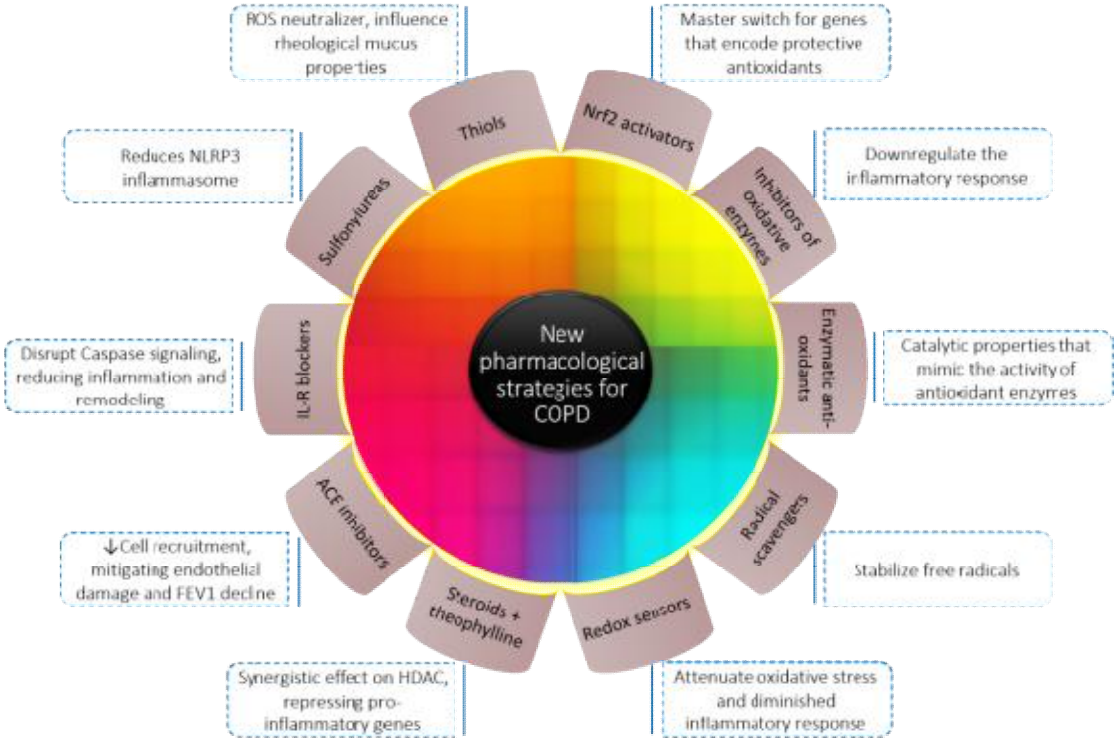

Fig. 4. Summary of different new pharmacological strategies for COPD

8.2 Anti-inflammatory Strategies

Inhaled glucocorticoids in usual dosage act by activating HDAC-2, which represses the activity of pro-inflammatory genes [54]. COPD patients have decreased expression and activity of HDAC-2 in airways, alveolar macrophages and lung parenchymal cells (due to nitration and oxidation). Hence, there is little substrate for glucocorticoids to act. In fact, glucocorticoids do not reduce the quantity of inflammatory cells, cytokines or proteases in the sputum of COPD patients. Glucocorticoids not only do not suppress neutrophilic inflammation, but inhibit neutrophil apoptosis [55]. Glucocorticoid treatment has no effect on the progression of the disease or its mortality. It has been reported a potential but slight reduction in exacerbations, although this finding has recently been questioned [56-58].

One mechanism of action of theophylline is the inhibition of PI3K-δ, molecularly this will reduce the effect of oxidative stress on HDAC-2, then increasing its expression and activity. In fact, low dose theophylline decreases MPO and sputum eosinophilia (inflammation markers) [59]. As mentioned, HDAC -2 is the substrate on which low-dose inhaled steroids exert their anti-inflammatory effect [54]. This could imply a synergistic effect between the two anti-inflammatory drugs, clinically effective and relatively inexpensive [59].

Angiotensin II (ANGII) is produced by the action of angiotensin converting enzyme (ACE) on Angiotensin I; ACE is present in high concentrations in the lung. ANGII has pro-inflammatory effects, as a recruiter of inflammatory and immune cells. It has been postulated that ANGII may contribute to the FEV1 decline in persistent smokers [60]. ACE inhibitors might therefore reduce the FEV1 declining phenomenon, and could mitigate the progressive endothelial damage that comes from the inflammatory process.

Even subjects with mild COPD have endothelial inflammatory infiltration and vascular remodeling. Patients with COPD show microalbuminuria, an indicator of systemic vascular endothelial dysfunction. It is possible that ACE inhibitors could have an effect on endothelial dysfunction and on vascular and parenchymal destruction. ACE inhibitors seem to be more effective in aging patients, patients with low BMI and rapid

FEV1 decliners (>30 cc/year). The protective effect of ACE inhibition also appears to be greater in patients with associated cardiovascular disease, hypertension and diabetes. But these results need to be validated in larger and more representative cohorts [61].

Cigarette smoke activates Caspase-1 and -11, these activate the IL–18 pro-peptide, which acts on its receptor. This process is a critical signal to induce inflammation and remodeling [62]. Blockage of IL-18 receptors, IL-1Rα (with Anakinra), IL-1β (with Trap/rilonacept), are possible treatment options for lung inflammation that leads to emphysema [25,62]. The sulfonylurea Glyburide (widely used drug for treatment of diabetes mellitus type 2) reduces NLRP3 inflammasome and may be useful in the treatment of inflammatory lung diseases [63].

8.3 Antioxidant Alternatives

8.3.1 Small molecule thiol antioxidants

N-acetyl cysteine (NAC) has direct and indirect properties as an antioxidant in the treatment of COPD. NAC free -thiol group is capable of interacting with the electrophilic groups of ROS. Indirectly, NAC is a precursor of GHS, a neutralizer of ROS. NAC may serve as a protective factor against internal and external ROS [64]. The largest trial of an antioxidant in COPD, the BRONCUS study, failed to show any effect of orally administered NAC in reducing progression of the disease or frequency of exacerbations. Although, there was an apparent benefit in COPD patients treated with NAC but not with inhaled steroids [65].

Study's results could be justified by NAC failure to act on the subcellular compartment, but could also be explained by insufficient drug dosage or frequency. One Israeli study found beneficial effects of NAC on trapped gas [66]. The recent HIACE study (1200 mg/day orally NAC for one year), showed significant improvement in small airway function and reduced frequency of exacerbations. But these studies need to be validated with larger caseloads [67].

Other -thiol antioxidants (such as carbo-cysteine) influence the rheological properties of mucus, increasing content of sialomucin and mucociliary clearance, and decreasing the activity of IL-6. The PEACE study showed that patients treated with carbo-cysteine presented less exacerbations than the placebo group, and were not receiving corticosteroids [68]. The EQUALIFE study, using another -thiol antioxidant, erdosteine, showed similar results [69].

8.3.2 Nrf2 activators

Perhaps the most encouraging approaches to antioxidant therapy lie with the new Nrf2 activators, which may also prevent oxidative stress-induced autoimmunity [31]. Nrf2 regulates almost all the antioxidants and phase II cytoprotective genes [70]. Within Nrf2 activators are the sulforophanes, phytodrugs present in broccoli and cruciform vegetables; they do activate Nrf2, but not always very potently. New Nrf2 activators are significantly more potent than sulforaphanes. Despite their great potential in COPD, Nrf2 activating drugs present possible concerning safety issues [71,72].

8.3.3 Inhibitors of oxidative enzymes

The inhibition of oxidative enzymes could be a promising approach. Xanthine oxidoreductase has been implicated in the development of oxidative tissue damage in a number of cardiovascular and respiratory disorders. XO level is 4 times higher in the sputum and BAL of patients with COPD, than in healthy controls. The use of XO inhibitors, such as allopurinol and febuxostat, may offer some benefit [73]. Celestrol (an active compound from the medicinal plant *Tripterygium wilfordii*) inhibits the four isoforms of NOX (NADP oxidase), and has a potential use in inflammatory diseases [74].

AZ1 (a MPO 2-thioxanthine inhibitor) appears to downregulate the inflammatory response induced by tobacco smoke, with the advantage that the inhibition is irreversible (i.e. suicide inhibitor). This could be of benefit in COPD, since most of the current inhibitors are short-acting or reversible [75]. Use of enzyme inhibitors requires confirmatory, well designed, large-scale studies to be considered as a therapeutic intervention in COPD [73].

8.3.4 Radical scavengers

Edaravone is a powerful free radical neutralizer, it reduces carbonyl stress and lipid peroxidation; therefore, it has a potential future in COPD treatment. Lazaroids are non-glucocorticoid-methylprednisolone analogues that penetrate hydrophobic membrane regions and inhibit lipid peroxidation. Their protective effects have been reported in animal models of lung injury, both

require studies to assess their potential in COPD. Spin traps are compounds which can stabilize free radicals, to form stable end products. Spin traps have been widely used for *in vitro* studies and their therapeutic effects have also been investigated in *in vivo* models of lung disease, some have been shown to have benefits [70].

8.3.5 Enzymatic antioxidants

Enzymatic antioxidants are small molecules with catalytic properties that can mimic the activity of antioxidant enzymes [70]. Those who resemble SOD (i.e. SOD mimetic) are the macrocyclic ligands based on manganese, manganese-metalloporphyrins and salens (which also block nitrative stress). The development of SOD-like molecules seems to be a rational therapeutic intervention in emphysema [76].

Ebselen (an organic selenium-based compound) neutralizes oxidative and nitrative stress, by resembling glutathione peroxidase activity. No reports are available as the beneficial effect of Ebselen against cigarette smoke-induced lung inflammation; although it has been shown to prevent LPS-induced lung inflammation in animal models [70]. Inhibition of inducible nitric oxide-synthase (iNOS) by various chemical complexes may provide a strategy in COPD management [77].

8.3.6 Enzymatic redox sensors

Thioredixin (Trx) and redox effector factor-1 (Ref-1), belong to the oxidoreductase family of redox sensors. Trx inhibition resulted in diminished neutrophil influx and TNF-α production in an animal model. Activation of Trx can attenuate oxidative stress. Trx effects in COPD remain to be investigated [70,78].

8.4 Not Approved Treatment

Nedocromil and leukotriene modifiers have not been adequately investigated in COPD and their use is not recommended. There is no evidence of benefit with infliximab (anti TNF-α) in the treatment of moderate to severe COPD; on the contrary, there is some evidence of infection and malignancy risk, two comorbidities already associated with COPD [79]. Herbal medicine has no evidence of effectiveness. Other options, such as homeopathy and acupuncture, have not been adequately evaluated [4].

Prophylactic usage of antibiotics is not indicated, because the risk-benefit ratio is unfavorable [80,81]. Antibiotics should only be used in infectious exacerbations or other bacterial infections in COPD.

9. CONCLUSIONS

High level of ROS initiates the inflammatory process in COPD, with a highly specific cellular and molecular profile. Through protease/anti-protease imbalance and diverse kinds of stress, the pathological phenomena involving the bronchopulmonary, vascular and systemic compartment is generated.

Autoimmunity and repeated infections potentially perpetuate and amplify the inflammatory process, even if the initial stimulus is suspended.

Failure of current bronchodilator and steroid therapies to attenuate natural evolution of the disease and progressive deterioration, indicates the need to develop powerful new drugs with innovative effects.

It is likely than drug combinations are required, instead of monotherapy, in order to improve the effectiveness and impact the progressive course and mortality of COPD.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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