Review

ANTIINFLAMMATORY PROPERTIES OF AMBROXOL

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Abstract

Ambroxol is frequently used as mucolytic agent in respiratory diseases associated with increased mucus production like acute or chronic bronchitis. Further, ambroxol is used topically (lozenges) for the treatment of sore throat and pharyngitis associated with common cold. In addition to the effects of ambroxol on mucus regulation and local anaesthetic effects, a wide range of pharmacological antiinflammatory properties of ambroxol have been described *in vitro* and *in vivo*, including inhibition or scavenging of oxidative and nitrosative stress, increase of local defense molecules involved in respiratory virus replication, reduction of proinflammatory cytokines and arachidonic acid metabolites, inflammatory cell chemotaxis, and lipid peroxidation of tissues. The present review summarizes the antiinflammatory effects of ambroxol and relates these properties to results from controlled clinical trials in targeted diseases such as chronic bronchitis, chronic obstructive pulmonary disease and sore throat.

Key words: Ambroxol, inflammation, COPD, bronchitis, cytokines, sore throat

BACKGROUND

AMBROXOL

Ambroxol, (2-amino-3,5-dibromo-N-(trans-4-hydroxy-cyclohexyl)benzylamine), a bromhexin metabolite, is widely used for the treatment of acute or chronic respiratory diseases associated with increased mucus production, such as chronic bronchitis. Due to its ability to promote bronchial secretion and clearance, ambroxol is used as a mucolytic or expectorant. Further, ambroxol has been used for the prophylaxis or treatment of respiratory distress syndrome, bronchopulmonary dysplasia, alveolar proteinosis, and postoperative pulmonary complications after major surgery [1, 2, 3, 4, 5]. More recently, a topical application of ambroxol (ambroxol lozenges) has been approved for the treatment of sore throat and pharyngitis associated with acute oropharyngeal infections. The pharmacological effects of ambroxol cover a wide range, including mucus regulation on gland cells, increased production of pulmonary surfactant, neutralization of oxidative and nitrosative stress, suppression of respiratory virus replication, reduction of proinflammatory cytokines, chemotaxis, respiratory burst of inflammatory cells, and tissue lipid peroxidation, as well as, noticeably, local anaesthetic effects.

In particular when used topically, some of the clinical effects seen with ambroxol, namely reduction of pain and swelling, appear to be attributable to local anaesthetic properties of ambroxol [6]. However, there is also evidence to suggest that the antiinflammatory properties of ambroxol play a major role in its effects on sore throat and pharyngitis, as well as interaction of ambroxol with viral replication per se. This review summarizes the anti-inflammatory properties of ambroxol (Table 1).

Table 1. Summary of antiinflammatory effects of ambroxol in the literature.

Mediator	Ambroxol effect	Reference
LTB_4	↓ release *in vitro*	17
cys-LT	↓ release *in vitro*	17
IL-1	↓ expression and secretion *in vitro*	27, 30
IL-2	↓ release *in vitro*	28
IL-4	↓ release *in vitro*	17
IL-6	↓ release and concentration *in vitro / in vivo*	22, 29, 30
IL-8	↓ release and concentration *in vitro / in vivo*	21, 22
IL-12	↓ release *in vivo*	47
IL-13	↓ release *in vitro*	17
IFN-γ	↓ release *in vitro*	28, 47
TNF-α	↓ secretion *in vitro*	27, 28, 30, 47
Histamine	↓ release *in vitro*	17, 24
Oxidative metabolites	↓ release *in vitro / in vivo*	30, 39, 40, 41, 42
Surfactant proteins	increased concentration and expression *in vivo*	46, 47

ANTIINFLAMMATORY EFFECTS OF AMBROXOL

Inflammation is generally defined as a distinct response of the immune system to noxious stimuli. Recruitment of inflammatory cells involves a series of regulated events including chemotactic stimuli like cytokines or lipid mediators, transendothelial migration of leukocytes through selectin-mediated tethering, rolling and subsequent adhesion by integrin-dependent mechanisms [7, 8]. At the respective site of inflammation, inflammatory cells become activated through multiple stimuli, which lead to release of proteolytic enzymes, cytokines, nitric oxide and superoxide anion. In inflamed tissues, clearing of inflammatory cells may also be delayed by survival-promoting factors, such as granulocyte-macrophage colony stimulating factor [9] or leukotrienes.

While the physiological role of the inflammatory response is generally to eliminate potential noxious agents or stimuli, the cellular response itself may act as a stimulus for inflammation to persist, thus forming a vicious cycle in such chronic inflammatory diseases, even after the initial trigger has been eliminated. However, this phenomenon may also be observed in acute disease states such as the systemic inflammatory response syndrome (SIRS) or acute rhinovirus infection, where it has been shown that not infection or viral cytotoxicity per se, but rather the inflammatory response to viral infection causes the majority of tissue damage and – consecutively – symptoms [10]. Numerous studies have evaluated the effect of ambroxol on inflammatory mechanisms, and these involve initiation, amplification and persistence of inflammation.

EFFECTS OF AMBROXOL ON CHEMOATTRACTANTS

In acute inflammation, chemoattractants and/or cytokines with chemoattractant properties are the primary initiators of an inflammatory response. Among the various chemoattractants, the lipid mediator leukotriene (LT) B_4 is one of the most potent and important chemoattractants in acute responses, such as viral infections. LTB_4 exerts its main effects on monocytes / macrophages and neutrophils, which are key players in the initiation of various acute inflammatory diseases [11]. After binding to its neutrophil receptor, LTB_4 elicits calcium influx, transmembrane potential changes, degranulation, increased expression of the CD11b/CD18 adhesion molecule and, as a result, chemotaxis. In many chronic inflammatory diseases there is evidence of CD11b/CD18 upregulation on neutrophils together with increased levels of LTB_4 in serum or respiratory secretions [12, 13]. For example in COPD, LTB_4 accounts for a large part of the total neutrophil chemotactic activity of airway secretions [14, 15]. Interestingly, LTB_4 also inhibits neutrophil apoptosis which may delay the resolution of inflammation in tissues [16]. Epithelial cells, but also inflammatory cells like mast cells, basophils, eosinophils, macrophages/monocytes, and neutrophils themselves represent a major source of LTB_4. In models of acute inflammation, ambroxol effectively reduced the release of LTB_4 from monocytes and neutrophils after stimulation [17]. Further in this regard, Stockley et al. have shown that ambroxol at therapeutic concentrations inhibits the neutrophil chemotactic response to various chemoattractants [18].

Alongside LTB_4, interleukin (IL) -8, a CXC-chemokine, has potent neutrophil and macrophage chemoattractant activities [8, 19]. IL-8 is consistently associated with, in particular neutrophil dominated, inflammation, and IL-8 concentrations in tissues, blood or other biological samples are often directly correlated with neutrophilia. Epithelial cells and macrophages are major sources of IL-8, and IL-8 is released following a number of inflammatory stimuli [20]. The release of IL-8 from bronchial epithelial cells was reduced after pre treatment of cells with ambroxol [21]. A further investigation in subjects with COPD showed that blood and salivary IL-8 was reduced after 10 days treatment with ambroxol [22].

In addition to neutrophils and monocytes, other effector cells play an important role in certain types of inflammation, e.g. basophils and eosinophils in allergic conditions. These cells are particularily responsive to T(helper)2 or mast-cell derived chemoattractant cytokines like IL-4, IL-13, or IL-5, and chemotactic mediators like cysteinyl leukotrienes or vasoactive peptides (e.g. histamines) [23]. In a study by Gibbs et al., ambroxol reduced both basophil release of cys-leukotrienes, IL-4, IL-13 and histamine, while also decreasing mast cell release of histamine in human skin mast cells 17.Mast cell release of histamine was also reduced by ambroxol in a different study using human adenoids [24].

EFFECTS OF AMBROXOL ON CYTOKINES

In addition to chemotaxis, the initiation of the inflammatory cascade is mediated by a sequence of signalling events directing effector cells into sites of inflammation. Leukocytes tethering, rolling and transmigration along endothelial walls of blood vessels is mediated by cell adhesion molecules [25], and their expression is among other mechanisms induced by classical proinflammatory cytokines like IL-1β, IL-4, IL-6, IL-13 or tumor necrosis factor (TNF) [26]. The release or production of some of these cytokines has been shown to be reduced by ambroxol.

Bianchi et al. demonstrated a significant reduction of IL-1 secretion from lipopolysaccharide (LPS)-treated human macrophages *in vitro* by ambroxol [27]. In their study, it was also shown that ambroxol significantly inhibited IL-1β mRNA expression, indicating an effect not only on cytokine release, but also production. Similar effects were observed by Pfeifer et al. who investigated the effects of ambroxol on IL-2 release from human bronchoalveolar lavage (BAL) and peripheral blood mononuclear cells [28]. In addition to IL-2, interferon (IFN)-γ release was also reduced by ambroxol, indicating a more general antiinflammatory effect of ambroxol on T-cells.

In a rat model of LPS-induced acute lung injury, ambroxol also potently reduced proinflammatory cytokines IL-6 and TNF-alpha compared to saline-treated animals [29]. In this study, the inhibitory effect of ambroxol was comparable to dexamethasone. Salivary levels of IL-6 were also significantly reduced by am-

broxol treatment in COPD patients after 10 days 22. Jang et al. [30] reported that ambroxol decreased the production of IL-1β, IL-6, and TNF-alpha in alveolar macrophages activated by LPS, while also reducing the production of superoxide anion, hydrogen peroxide, and nitric oxide and the release of cellular granular enzymes like lysozyme.

TNF-alpha, besides its general function as proinflammatory cytokine, also promotes the chemotactic response of inflammatory cells to chemoattractants in various ways [19]. Therefore, it seems noticeable in this regard, that Bianchi et al. demonstrated an *in vitro* suppression of approximately 90% by ambroxol on the production of TNF from human macrophages after stimulation with lipopolysaccharide (LPS) [27]. TNF production by BAL cells and peripheral blood mononuclear cells PBMCs was also significantly reduced by ambroxol in another study by Pfeifer et al. [28].

Finally, Aihara et al. studied the effect of ambroxol on LPS-induced secretion of IL-12 and IL-10 by human alveolar macrophages [31]. In particular, they investigated the ratio of IL-12/IL-10, since it is assumed that the particular ratio of these cytokines regulates T-cell responses rather than their mere concentrations per se. In their study, ambroxol increased the secretion of IL-12, but not IL-10, thus shifting the IL-12/IL-10 ratio in favour of IL-12. This observation would indicate a possible role of ambroxol in enhancement of T-cell mediated immunity. However, one needs to take into account that macrophage-derived TNF negatively regulates IL-12 secretion of macrophages, thus the observed increase of IL-12 secretion could also be a direct consequence of the previously described inhibitory effect of ambroxol on TNF-secretion.

The mechanisms by which ambroxol elicits antiinflammatory effects on release and production of various cytokines are as yet incompletely understood. One possible explanation is a direct inhibition of phosphodiesterases (PDE), including PDE-4, by ambroxol, as reported by Ferretti et al. [32]. Moreover, ambroxol could also interact with the transcription factor NF-kappa B, thus reducing intracellular production of proinflammatory cytokines [21]. Finally, Kim et al. observed an inhibition of cellular activation processes involving proteine kinase C and protein tyrosine kinases by ambroxol [33].

OXIDATIVE AND NITROSATIVE STRESS

Oxidative stress results from the increased presence and activation of inflammatory cells, in particular neutrophils, which generate vast amounts of reactive oxygen intermediates as part of their innate antibacterial defense. Several cellular and non-cellular defense mechanisms protect resident cells from injury due to oxidative stress. However, in chronic inflammatory conditions, there is ample evidence that endogenous defense mechanisms are insufficient to counteract oxidative injury [34-37]. Inflammatory cell-derived oxidants can also interact with other cellular mediators to enhance their cytotoxic effects. For example, neutrophil-derived myeloperoxidase (MPO) and hydrogen

peroxide (H₂O₂) promote both epithelial and parenchymal tissue damage through formation of toxic hypochlorous acides (HClO), activation and increased release of cytokines [38]. Further, the interaction of reactive oxygen intermediates with nitric oxide (NO) metabolites generated by NO-synthases (NOS), which are activated or induced by proinflammatory cytokines, promotes the formation of highly reactive nitrogen species, in particular peroxynitrite (ONOO-). Therefore, the restoration of the oxidant/antioxidant imbalance and counteraction of nitrosative stress has been a desirable therapeutic option in various chronic inflammatory diseases.

Ambroxol has been shown to have beneficial therapeutic effects on markers of oxidative stress in a number of investigations. Gillissen et al. showed that ambroxol at therapeutic concentrations reduced the release of reactive oxygen species (ROS) by polymorphonuclear cells in a time-dependent manner, suggesting that ambroxol did not only have the potential to directly scavenge free radicals, but also alter the prooxidative metabolism in inflammatory cells [39]. Further, Stetinova et al. demonstrated that ambroxol inhibited hyaluronic acid degradation induced by hydroxy radicals and lipid peroxidation by hydroperoxide both *in vitro* and *in vivo* [40]. Ambroxol also inhibited peroxynitrite- and hypochlorous acid induced damage of alpha-1—antiproteinase, an important endogenous inactivator of neutrophil-derived tissue degrading elastase, in a study by Lee et al. [41]. In the same study by Lee et al., ambroxol also significantly reduced the production of superoxide, hydrogen peroxide, HClO, and nitric oxide in IL-1 activated phagocytic cells. These observations have also been confirmed by Jang et al. [30] and Ottonello et al. using activated human neutrophils [42].

ANTIVIRAL EFFECTS OF AMBROXOL

Although ambroxol is often used in the treatment of acute upper and lower respiratory tract infection, few studies have investigated the direct effect of ambroxol on infectious agents, e.g. human respiratory viruses. Acute viral respiratory infections are mainly caused by rhinovirus (30-50%), corona virus (10-15%), parainfluenza (5%) and respiratory syncytial virus (5%) [43]. While studies indicate that cytopathic effects of rhinovirus on epithelial cells are weak and neutrophilic inflammatory infiltrates appear relatively mild [44], adenoviruses and, in particular, influenza A virus cause significant epithelial damage in the human respiratory tract. Entry and replication of influenza A in respiratory cells is facilitated by epithelial proteases, e.g. trypsin-like protease, or tryptase clara, which interact with viral envelope membrane glycoproteins, thus further promoting viral cell entry. These host proteases in turn underly regulation by endogenous cellular suppressors, such as secretory leukoprotease inhibitor, human mucus protease inhibitor, or, in the lower airways, pulmonary surfactant [45]. Hence, upregulation of natural inhibitors of proteases represents a potential therapeutic approach to suppress viral airway replication. Seifart et al. studied the effect of ambroxol on surfactant proteins in rats. In their investigation, am-

broxol increased protein and mRNA content of pneumocytes or clara cells of surfactant proteins -C and -D [46]. Yang et al. demonstrated also in rats, that treatment of animals with increasing concentrations of ambroxol prior to infection with influenza A virus caused a significant suppression of virus multiplication and improved overall survival [47]. This beneficial effect was accompanied by an increase of virus replication inhibitors mucus protease inhibitor, surfactant protein A, and immunoglobulines –A and –G, while ambroxol simultaneously reduced inflammatory cytokines TNF-alpha, IFN-γ, and IL-12. The mechanisms underlying the stimulation of immunoglobulines and inhibitory proteins by ambroxol are, again, not understood. However, the observed effect on several target cells in the upper and lower airways again suggest a more general effect on central regulatory cellular mechanisms, like the PDEs or NF-kappa B as discussed above. Interestingly, a protective effect of ambroxol on the number of acute upper respiratory disease episodes was recently demonstrated in a controlled clinical trial [48].

Clinical Studies with Ambroxol Supporting an Antiinflammatory Effect *in vivo*

Numerous clinical studies have been performed with ambroxol, either in its oral, inhaled, or topical preparation. Indications for therapeutic long-term use included asthma, chronic bronchitis / COPD, and viral infections / common cold, in particular sore throat.

In asthmatic subjects, ambroxol reduced airway reactivity to methacholine after 14 days of oral treatment (90 mg) [49]. However, ambroxol had no effect on the cough threshold to capsaicin in asthmatics with cough-variant asthma [50]. To date, no long-term studies have evaluated a potential efefct of ambroxol in the treatment of asthma.

In chronic bronchitis and / or COPD, Poole et al. described a protective effect of mucolytics usage including ambroxol on the overall frequency of exacerbations, by means of a systematic review [51]. The meta-analysis included a placebo-controlled clinical study by Olivieri et al. who demonstrated an increase of exacerbation free intervals with ambroxol [45]. These observations were – at least partially – supported by a placebo-controlled prospective trial using ambroxol 75 mg twice daily for one year in subjects with COPD (AMETHIST-Trial) [52]. Although a beneficial effect of ambroxol in the overall group was not found, those patients with more severe COPD experienced significantly fewer exacerbations with ambroxol compared to placebo. This finding concurs with observations from controlled clinical trials studying the effect of inhaled steroids on exacerbations in COPD, where the effect also appears to be restricted to patients with more severe COPD and a history of recurrent exacerbations [53]. A further investigation in subjects with COPD showed that blood and salivary IL-8 was reduced after 10 days treatment with ambroxol [22], indicating an underlying antiinflammatory effect..

Since many exacerbations in COPD are virally triggered [54, 55], a further possible explanation for an effect of ambroxol on COPD exacerbations may be the preventive effect on acute viral upper respiratory tract infections [48].

More recently, a topical preparation of ambroxol was introduced into the treatment of pharyngitis and sore throat associated with acute viral cold. In a proof-of-concept trial, 20 mg ambroxol lozenges led to a reduction of sore throat pain superior to placebo in 218 patients [56]. In two further confirmatory trials, 20 or 30 mg of ambroxol also relieved pain in sore throat over placebo, although the higher dose was no more effective than the lower dose [57]. Although local anaesthetic effects for ambroxol have been described [6], the reduction of mucosal hyperemia and redness observed in clinical studies suggests, that the clinical benefits of ambroxol lozenges in sore throat are at least partially mediated by antiinflammatory properties of ambroxol (de Mey C, Peil H., Koelsch, S., Bubeck J., Vix J.M., EBM-based clinical documentation of the efficacy and safety of lozenges containing ambroxol in treating acute uncomplicated sore throat, *manuscript in preparation*).

Summary and Conclusion

Ambroxol is widely used as a mucolytic in respiratory diseases associated with increased mucus production, e.g. acute or chronic bronchitis. Further, ambroxol is used topically for the treatment of sore throat and pharyngitis associated with common cold. In addition to the effects of ambroxol on mucus regulation and local anaesthetic effects, a wide range of pharmacological antiinflammatory properties of ambroxol have been described *in vitro* and *in vivo*, including inhibition or scavenging of oxidative and nitrosative stress, increase of local defense molecules involved in respiratory virus replication, reduction of proinflammatory cytokines and arachidonic acid metabolites, inflammatory cell chemotaxis, and lipid peroxidation of tissues, while some of these antiinflammatory effects are also supported by results from controlled clinical trials, e.g. in chronic bronchitis / COPD and virally induced sore throat.

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