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MINI-REVIEW

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# Amlexanox: A Novel Therapeutic for Atopic, Metabolic, and Inflammatory Disease

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Amlexanox, a small molecule targeted therapy which has been used in the treatment of atopic conditions was previously but is not currently available in the United States. Amlexanox has also been legally utilized and administered in Japan as a treatment for asthma, a chronic pulmonary disease characterized by inflammation of the lower respiratory tract. Amlexanox's immune modulatory effects have been the subject of studies which have repurposed the drug for potential therapeutic applications in metabolic and inflammatory disease. Because amlexanox inhibits TANK-binding kinase1 (TBK1) and nuclear factor kB kinase epsilon (IKKε), several studies have demonstrated its usefulness through its evidence downregulation of the immune system and attenuation of downstream TBK1 signaling. Novel therapies, such as amlexanox, for inflammatory conditions such as asthma will continue to be of value in clinical management. This report summarizes key applications of the drug based on animal and human studies and explores its potential in treatment of metabolic and inflammatory diseases.

## INTRODUCTION

Amlexanox (trade name Solfa), a tricyclic amine carboxylic acid, is a potential treatment option that was traditionally used as a treatment of recurrent aphthous ulcers until its discontinuation in the United States in 2017, as other options became available [1]. In other countries, such as Japan, amlexanox is authorized to treat asthma, allergic rhinitis, and conjunctivitis [2]. Amlexanox has also been shown to treat and reverse the progression of fatty liver disease (NAFLD) in a promising study conducted by He *et al.,* which utilized a mouse model and demonstrated improvement in metabolic disturbance and hepatic steatosis [3]. The purpose of this review is to examine its potential as a therapeutic immunomodulatory agent for metabolic conditions and inflammatory diseases, based on existing novel studies and current uses for amlexanox.

## DRUG PROFILE

Amlexanox (chemical structure $C_{16}H_{14}N_2O_4$) is a stable compound as a powder stored at -20 °C with a shelf life of 3 years. It has a similar chemical structure to sodium cromoglycate (SCG). Its topical use has been widely

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Abbreviations: NAFLD, fatty liver disease; TBK1, TANK-binding kinase1; cAMP, cyclic-AMP; AIA, aspirin-induced asthma; SCG, sodium cromoglycate; cGAMP, cyclic GMP-AMP; cGAS, cyclic GMP-AMP synthase; IFN, interferon; STING, stimulator of interferon genes; HDM, house dust mite.

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accepted and its effects attributed to anti-inflammatory qualities. The successful use of this treatment with a favorable safety profile in atopic disease as an oral agent has been established and is currently approved for the treatment of asthma in Japan.

The efficacy and safety profile of amlexanox was previously evaluated by a number of studies. Of note, a 6-month toxicology study using dogs determined that the dosage of oral amlexanox at which no side effect is demonstrated is 10 mg/kg/day. It was later determined that the dosage for the amlexanox paste for ulcers would be approximately 12 mg/day of amlexanox. Furthermore, it was demonstrated that when amlexanox is administered to rats at a dosage of 300 mg/kg/day, there were no carcinogenic effects and had no significant effect on reproductive activity on rats [4].

The main contraindication for the administration of amlexanox is any known hypersensitivity to amlexanox itself or other ingredients found in the formulation. A case report of a 23-year-old female who was treated for allergic rhinitis with oral Solfa 25 mg describes an eruptive skin rash during treatment. The positive patch test based on a suspected allergic localized skin lesions confirmed an allergic reaction to amlexanox [5].

Based on a double-blind randomized placebo-controlled study of 42 obese subjects, there were no serious significant adverse events [6] to 3 times daily oral amlexanox. In the study, two patients who experienced a perivascular inflammatory rash improved with local treatment and both had a favorable response to the drug.

Given the recent data that demonstrates the potential for amlexanox, a TANK-binding kinase1 (TBK1) inhibitor, as a treatment to be further explored to reduce inflammation in a wide range of conditions, including metabolic syndrome, Type 2 diabetes, and NAFLD.

## MECHANISM OF ACTION

There is evidence of its role as an anti-inflammatory mediator and antihistamine [7]. Among its cellular effects, the drug is an inhibitor of nuclear factor kB kinase epsilon (IKKε) and TBK1. This property led to further scientific investigations in molecular, cellular, animal models, and human studies, which have been summarized in Table 1. A study published in 2019 by Quan *et al*. provides evidence that amlexanox inhibits TBK1, thus inhibiting dendritic cell maturation and decreasing inflammation caused by the innate immune response on a standard mouse model [8]. They identified that in the presence of amlexanox, in the context of autoimmune encephalitis, phosphorylation of IRF3 and AKT, two downstream targets of TBK1, were decreased. Furthermore, amlexanox has been identified to bind to fibroblast growth factor 1 (FGF-1), a mitogen which is released when cells undergo stress [9].

A novel study conducted in 2019 demonstrated that amlexanox, when administered both intranasally and orally, can alleviate the amount of host-derived DNA, which is released from neutrophils and eosinophils when the cyclic GMP-AMP (cGAMP) is activated [10]. The cell can recognize the host-derived DNA by cytosolic cyclic GMP-AMP synthase (cGAS) and then recruits stimulator of interferon (IFN) genes (STING) at its membrane, leading to the activation of and phosphorylation by TBK-1 [10]. Intranasal amlexanox was administered to 6-week-old mice, in a cGAMP-adjuvanted model of house dust mite (HDM) airway inflammation. After administration of the TBK1 small molecule targeted therapy, the drug was associated with amelioration of the allergic responses. Specifically, eosinophil recruitment, serum total IgE, and HDM-specific IgG responses were determined in mice lungs as a measure of immune response. The data demonstrates that mice who received amlexanox had a significantly decreased immune cell response and cell recruitment in the lungs. The authors reported that TBK1 is needed for the induction of IL-33. They also found that IRF3/7, which are signal transducers downstream of TBK1, are necessary for IL-33 extracellular release from lung fibroblasts in response to cGAMP. The authors concluded that the TBK1 inhibitors are a promising class of drugs in the treatment of asthma. While this study is a mouse model, there was a significant reduction in the markers of allergic inflammation and a cellular mechanism identified which identifies a therapeutic target in asthma.

Based on a previous study conducted by Makino *et al*. in 1987, amlexanox decreases allergic responses by inhibiting histamine release from mast cells [11]. It was observed that by inhibiting cyclic-AMP (cAMP) phosphodiesterase in rat mast cells, intracellular cAMP levels were increased, which inhibited the release of histamine. Furthermore, a study conducted by Gonzalez-Hilarion et *al.* demonstrates that amlexanox also holds a dual function in patient cell lines that have nonsense mutations. Amlexanox has been found to not only increase nonsense containing mRNAs, but also leads to synthesis of a full-length functional protein, which thus prevents nonsense-mediated mRNA decay (NMD) [12].

## CURRENT USES OF AMLEXANOX IN TREATMENT OF ASTHMA

Amlexanox has been authorized to treat asthma in Japan since 1987, and it is administered as an oral tablet. Amlexanox is currently produced in either 25 mg or 50 mg tablets in Japan by Takeda Pharmaceuticals for the treatment of allergy-induced asthma (Takeda Pharmaceuticals, 1993).[1] Previous studies have demonstrated its ef-

**Table 1. Summary of Key Novel Studies on Amlexanox Conducted.**

| Authors | Study Design | Pertinent Results | Takeaways |
|---|---|---|---|
| Gonzalez-Hilarion *et al* (2012) [12] | Utilized screening to determine small molecules which can act as potential inhibitors.<br><br>Administered to 3 derived cell lines with nonsense mutation and observed results. | Determined an increase in nonsense containing mRNAs in treated cells.<br><br>Synthesis of full length, functional protein.<br><br>Evidence that amlexanox rescues expression of nonsense-containing mRNAs. | Amlexanox should be further investigated to determine its effect on diseases caused by nonsense mutations. Has effect on potential conditions such as cystic fibrosis. |
| Oral *et al* (2017) [6] | Double-blind, placebo study of 42 obese patients with type 2 diabetes and nonalcoholic fatty liver disease (NAFLD). Length: 12 weeks. | Patients administered amlexanox demonstrated improved insulin sensitivity and hepatic steatosis.<br><br>Increase in serum IL-6 levels at 2-4 weeks. | Amlexanox to be further examined as a potential therapeutic for NAFLD and type 2 diabetes. |
| Beyett *et al* (2018) [24] | Utilized both amlexanox and derived analogs to determine cellular effect on inflammation. | Determined that amlexanox likely inhibits kinases which affect chronic low-grade inflammation. | Amlexanox is promising therapeutic in treatment of type 2 diabetes and obesity through its presumed mechanism of action. |
| He *et al* (2019) [3] | NAFLD mice models were established using 8-week-old mice, which were fed either high-fat diet (HFD) and/or lipopolysaccharide (LPS) diets. All HFD mice administered either amlexanox or vehicle for 18 weeks. HFD+LPS mice were administered either amlexanox or vehicle for the final 6 weeks. | Amlexanox improved insulin signaling in hepatocytes through inhibiting inflammation in hepatic stellate cells (HSCs). IKKe was detected only in HSCs and was not identified in hepatocytes. | Amlexanox has the potential to improve the insulin signaling pathway in hepatocytes, which further demonstrates promise as a future therapeutic for metabolic disease. |

ficacy in treating asthma and allergic reactions, including allergic rhinitis. The drug treatment, based on its inhibition of release of histamine and inflammatory leukotriene mediators by mast cells, neutrophils, and monocytes, has been recommended based on Japanese treatment guidelines for allergic rhinitis [13].

In human studies, a study conducted by Imokawa *et al*. demonstrated that amlexanox acts as a bronchodilator for patients with aspirin-induced asthma (AIA). Amlexanox has a similar chemical structure to sodium cromoglycate (SCG), which is a known bronchodilator used to treat asthmatic patients (Imokawa, 1993 [14]). In this study of 15 patients, 7 non-aspirin induced, and 8 aspirin induced asthmatics were studied. Spirometry was performed at regular time intervals following administration of either the drug or placebo. The aspirin induced group improved their overall FEV1 following administration of the drug. While the other asthmatics did not improve over the time course of 3 hours, its use in this population should be further evaluated, given its effect as a bronchodilator. The authors concluded that the aspirin induced asthmatics benefited from the use of amlexanox.

According to the 2017 Japanese guidelines for adult asthma, amlexanox is currently identified as a reliever agent, a "rescue agent aimed at treating asthma exacerbations," due to its histamine-inhibiting properties. It is often prescribed in addition to controller agents, which are prescribed to control the long-term symptoms of asthma [7]. The authors classify the drug as a "mediator anti releaser." The clinical effect of this drug in improving asthmatic control extend beyond its antihistamine properties.

## POTENTIAL THERAPEUTIC EFFECTS FOR METABOLIC AND LIVER DISEASE

While the mechanisms are not fully elucidated, the repurposing of a drug used in the treatment of inflammation of the skin and lungs to metabolic and liver disease warrants further investigation.

In addition to its use as a treatment for asthma, am-

lexanox has been explored in multiple settings, including diabetes, obesity, and liver disease. Much has been identified about the role of TBK1 with insulin-stimulated glucose uptake, which may be linked to diabetes, obesity, and inflammation. A study conducted by Uhm *et al.* identified that upon activation by RalA, TBK1 phosphorylated an exocyst protein Exo84, which translocated the GLUT4 glucose transporter to the cell membrane [15]. TBK1 has also been noted to phosphorylate an insulin receptor and in a study of obese Zucker rats, Munoz *et al.* observed an increased association between TBK1 and insulin receptor phosphorylation, which indicates a potential link between insulin resistance and TBK1 [16]. Furthermore, a study conducted by Zhao *et al.* determined that in animals fed a high-fat diet, knocking out TBK1 prevented the development of high fat diet driven obesity. As a result, the researchers hypothesized that TBK1 phosphorylated AMP-activated protein kinase (AMPK) activity, which further increased energy storage [17]. These notable studies critically reveal the role of TBK1 in driving inflammation and disrupting metabolism.

The cellular effects of amlexanox led to investigation of its use in other inflammatory conditions. Specifically, other studies have been conducted to examine the impact of amlexanox in ameliorating obesity-related metabolic dysfunctions. In an experimental animal model of obesity, amlexanox was administered to examine its effects on cytokine signaling, specifically in IL-6 production. Because amlexanox inhibits TBK-1, this evidence potentially points to an indirect pathway in which amlexanox increases the secretion of IL-6 from adipocytes through a cAMP/ p38-dependent pathway. IL-6, in turn stimulates phosphorylation of hepatic STAT3 to suppress expression of gluconeogenesis in obese mice [18].

Two kinases, IKKe and TBK1, are linked causally to obesity related inflammation [19]. Downstream inflammatory consequences include the development of diabetes and fatty liver disease. In an animal model, the drug treatment improved insulin sensitivity and decreased steatosis and hepatic expression of inflammatory genes [20]. The inhibitor of IKKe and TBK1 kinases was studied in a proof of concept randomized double-blind, placebo-controlled study of 42 obese patients with type 2 diabetes and NAFLD. Treatment of patients produced a reduction of HbA1C, a measure of glucose attached to hemoglobin, of $\geq 0.5\%$ among 33% of the treated patients. The trial also showed that there was a reduction in fructosamine. Upon further analysis of responders, a distinct responder profile emerged. Those subjects who responded to amlexanox had higher baseline inflammatory markers in serum and in gene expression profiles. Among the responders to the 50 mg 3 times daily dosing, at 12 weeks, insulin sensitivity improved. Two subjects had a comorbidity of asthma/ emphysema, but the other subjects did not have asthma. The asthmatic subjects were not reported to have a differential response to treatment of their diabetes, compared to the other subjects. The further analysis of drug responses of this patient population with co-morbid asthma is a subject of future investigation. Other findings among the treatment group included increased expression of the gene encoding the B3-adrenergic receptor (ADRB3) [6]. Takeuchi *et al.* hypothesize that the polymorphisms in ADRB3 receptor are associated with obesity and decreased lipolysis, which makes this finding notable [21]. Interestingly, the use of the drug to treat asthmatics and improvement in asthma control may involve altered gene expression profiles, which are yet to be investigated.

## CONCLUSIONS AND OUTLOOK

Given the enormous range of potential effects, amlexanox should continue to be explored with regards to its immunomodulatory effects. A nearly obsolete asthma treatment may well hold promise as a therapeutic agent in the treatment of metabolic conditions such as Type II diabetes and obesity related NAFLD. This is critical, considering non-alcoholic fatty liver disease (NAFLD) is associated with obesity and afflicts an estimated 2-5% of Americans and is associated with cellular inflammatory infiltrates and liver fibrosis. The finding of NAFLD is part of other features of the metabolic syndrome such as obesity, diabetes mellitus 2, hypertension, and dyslipidemia [22], which call for serious investigation for potential therapeutics in order to curb the growing obesity epidemic.

Furthermore, despite numerous therapeutics that are authorized for use in the United States which ameliorate the symptoms of asthma, these have been associated with low adherence [23]. This may lead to poorly controlled asthma and increased exacerbations. Further exploration of amlexanox and potential reincorporation into the United States is warranted. Based on current data, amlexanox as an anti-inflammatory agent and antihistamine has been previously and successfully used in the treatment of asthma and atopic conditions. The targeted suppression of chronic inflammation in Type II diabetes, obesity, NAFLD, and metabolic dysfunction warrants further investigation. Given the data that demonstrates the potential for amlexanox, a TBK1/IKKe inhibitor, this expands potential future targeted immunomodulatory small molecule and should be further considered in the development of novel therapeutic agents to treat conditions such as asthma, atopy, and metabolic dysfunction.

Footnote

[1]Package insert, amlexanox (NJ): https://www.accessdata.fda.gov/drugsatfda_docs/label/2002/20511s002lbl.pdf, some data is gathered from the Takeda paper (original: Package insert for Solfa 25 mg and 50 mg tablets. Takeda Chemical Industries Ltd.

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