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(54) Title: COMPOSITIONS AND METHODS FOR AMELIORATING SJOGREN'S SYNDROME

(57) Abstract: Provided are compositions and methods of ameliorating Sjogren's Syndrome comprising administration of an effective amount of one or more GABA-receptor agonists, including GABA_A-receptor agonists, GABA_B-receptor agonists, GABA_{A- α 10} receptor agonists, and combinations thereof.

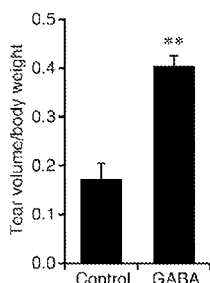


Figure 5C

COMPOSITIONS AND METHODS FOR AMELIORATING SJOGREN'S SYNDROME

STATEMENT OF FEDERALLY-SPONSORED RESEARCH

This invention was made with government support under Grant Number DE029020, awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Sjögren's syndrome ("SS") is a complex chronic autoimmune disease characterized by immune cell infiltration into exocrine glands, particularly the salivary and lacrimal glands which leads to a severe loss of secretory function and consequent xerostomia (dry mouth) and keratoconjunctivitis sicca (dry eyes) (1-4). The disease affects four million Americans with over 90% of those affected being female (5). The underlying etiology of SS is thought to be heterogeneous and remains elusive but is thought to involve abnormal salivary gland homeostasis and progressive tissue damage by infiltrating immune cells and autoantibodies. Analysis of human salivary glands and experimental mouse models of SS reveals that focal lymphocytic infiltrates are dominated by CD4⁺ T cells with B cells and macrophages increasing with disease progression (6-12). Experimental evidence points to Th17, Th1, Th2, and follicular helper T (Tfh) cells as contributors to the pathogenesis of SS (12-20). Additionally, several types of autoantibodies are associated with the development and progression of SS, including anti-nuclear, rheumatoid factor, anti-SSA/Ro, anti-SSB/La and anti-M3R autoantibodies (19).

Currently, treatments for SS are largely limited to ameliorating the disease's symptoms with artificial tears and mouthwash. Application of cyclosporine and rebamipide in eye drops has had beneficial effects in a limited number of SS patients, and clinical studies with antibodies that inhibit B and T cell activity, such as Rituximab, Belimumab and Abatacept, failed to reach the primary endpoints of efficacy (reviewed in (21)). Research efforts in animal models of SS have identified a number of treatments with therapeutic effects, but often their safety has not been established and they have not yet reached clinical testing. Accordingly, there is an urgent need to identify additional compounds, particularly those with an established safety profile, that could help ameliorate SS.

BRIEF SUMMARY OF THE INVENTION

Methods for ameliorating Sjogren's Syndrome, comprising administering to a patient in need thereof an effective amount of one or more GABA-receptor agonists. In certain embodiments, said GABA-receptor agonist is selected from the group consisting of: GABA_A-receptor agonists, GABA_B-receptor agonists, GABA_{A-rho} receptor agonists, and combinations thereof. Acceptable GABA-receptor agonists include GABA. When the GABA-receptor agonist is GABA, it may be administered in an amount of 0.001 gram/day to 30 grams/day, preferably 0.1 gram/day to 10 grams/day. An effective amount of an anti-inflammatory compound and/or a corticosteroid may also be administered.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Figure 1 shows the results of prophylactic treatment in NOD.B10-H2^b mice. Eighteen weeks old female NOD.B10-H2^b mice were placed on plain water, or GABA-containing water (6 mg/ml) for 14 weeks. At 32 weeks in age, the saliva flow, the time to saliva flow, and the basal tear production following isoproterenol and pilocarpine injection of the mice were analyzed, as well as the number of lymphocytic foci in their SMG and ELG. **A)** Saliva production. Data shown is mean amount of saliva $\pm$ SD collected over 15 minutes per gram body weight. **B)** Time to saliva production (minutes). **C)** Tear production. Data shown is mean $\pm$ SD of the length of thread wetting (mm) over 20 seconds (average of both eyes) per gram body weight at 10 minutes post- pilocarpine and isoproterenol injection. **D)** Mean number $\pm$ SD of lymphocytic foci in the SMG, **E)** mean area $\pm$ SD of infiltrates in the SMG, **F)** mean number $\pm$ SD of foci in the ELG, and **G)** mean area $\pm$ SD of infiltrates in the ELG of control and GABA-treated mice. For all data n=4 control and 6 GABA-treated mice. *p<0.05 and **p<0.01 by Students t-test.

Figure 2 shows that GABA treatment after the onset of symptoms preserves exocrine functions in NOD.B10-H2^b mice. Twenty-four-week-old NOD.B10-H2^b mice were placed on plain water (control) or water containing GABA (20 mg/ml) for 6 weeks. At 30 weeks in age, saliva flow and basal tear production was analyzed. Data shown are mean amount of saliva collected $\pm$ SD over 15 minutes post-isoproterenol and pilocarpine injection per gram body weight. **B)** Time to saliva production (minutes) following isoproterenol and pilocarpine injection. **C)** Tear production. Data shown are mean $\pm$ SD length of thread wetting (mm) over 20 seconds (average of both eyes) per gram body weight at 10 minutes post-pilocarpine

and isoproterenol. N=8 control and 9 GABA-treated mice. ** $p < 0.01$ and *** $p < 0.001$ by Students t-test.

Figure 3 shows the number of foci and area of infiltrates in SMG and ELG from NOD.B10-H2^b mice treated with GABA post-disease onset. **A)** Mean number of lymphocytic foci in the SMG, **B)** mean area of infiltrates in the SMG, **C)** mean number of foci in the ELG, and **D)** mean area of infiltrates in the ELG of control and GABA-treated mice. For SMG n=8 control and 9 GABA-treated mice. For ELG n=4 control mice and 5 GABA-treated mice. There was no significant difference between groups by Students t-test. Representative H&E stained images from **E)** control SMG, **F)** GABA-treated SMG, **G)** control ELG and **H)** GABA-treated ELG. Scale bars are 250 μm .

Figure 4 shows the measures of exocrine function in C57BL/6.NOD-Aec1Aec2 mice that received GABA prophylactically. **A)** Saliva production. **B)** Time to saliva flow (minutes) following isoproterenol and pilocarpine injection. **C)** Tear production. **D)** Mean number of lymphocytic foci in SMG and **E)** mean area of infiltrates in SMG. For panels A-E n=4 mice/group. **F)** Mean number of foci in ELG, and **G)** mean area of infiltrates in ELG of control and GABA-treated mice. For panels A-E n=4 mice/group, and for panels F-G n=4 control and 3 GABA-treated mice. None of the differences were significant by Students t-test.

Figure 5 shows that GABA administration after overt disease onset in C57BL/6.NOD-Aec1Aec2 mice preserves exocrine functions. Sixteen-week-old C57BL/6.NOD-Aec1Aec2 mice were placed on plain water or water containing GABA (20 mg/ml). At 20 weeks in age, we analyzed their exocrine function. **A)** Saliva production. The data shown are the mean amount of saliva produced per gram body weight. **B)** Tear production. The data shown are the mean length of

wetted thread per gram body weight. **C)** Time to saliva production. N=4 control and 5 GABA-treated mice. ** $p < 0.01$ by Student's t-test.

Figure 6 shows the number of foci and area of infiltrates in SMG and ELG from C57BL/6.NOD-Aec1Aec2 mice treated with GABA post-disease onset. The SMG and ELG from C57BL/6.NOD-Aec1Aec2 mice that were given plain water or GABA at 16 weeks in age were harvested at 20-24 weeks in age. **A)** Mean number of lymphocytic foci in SMG and **B)** mean area of infiltrates in SMG (n=9-10 mice/group). **C)** Mean number of foci in ELG, and **D)** mean area of infiltrates in ELG in control and GABA-treated mice (n=4 and 8 mice, respectively). Representative images of H&E stained sections from **E)** control SMG, **F)** GABA-treated SMG, **G)** control ELG, and **H)** GABA-treated ELG. Scale bars are 250 μm .

DETAILED DESCRIPTION OF THE INVENTION

It has been shown that many types of immune cells have receptors for the neurotransmitter GABA and that the activation of these receptors generally has anti-inflammatory activities (22-37). T cells express type A GABA-Rs (GABA_A-Rs) (22, 26, 29, 30, 36-39) and the oral administration of GABA or the GABA_A-R agonist homotaurine inhibits autoreactive CD4⁺ Th1 and Th17 cell as well as autoreactive CD8⁺ T cell responses, while simultaneously promoting CD4⁺ and CD8⁺ Treg responses (27, 29, 30, 40). The oral administration of GABA-R agonists ameliorates autoimmune disease in mouse models of type 1 diabetes (T1D), multiple sclerosis, and rheumatoid arthritis, and also limits inflammation in murine type 2 diabetes (23, 29, 30, 36, 38, 41). Recently, it was shown that GABA administration can limit the excessive inflammatory responses that cause pneumonitis and death in mice infected with a murine coronavirus (37). Thus, GABA-R agonists can inhibit disease

in different models of chronic autoimmune diseases, which have different etiologies and occur in mice with different genetic backgrounds. Human T cells and antigen-presenting cells also express GABA_A-Rs and their activities are regulated by GABA_A-R agonists and antagonists (39, 42-44). Notably, GABA inhibits secretion of IL-6, TNF, IL-17A, CXCL10/IP-10, CCL4, CCL20, and MCP-3 from anti-CD3 stimulated PBMC from T1D patients (39). Yet, GABA does not cause lymphopenia and leukopenia and is safe for human consumption, making it a promising candidate for ameliorating inflammatory disorders.

The inventor hypothesized that GABA treatment could be an effective therapy for ameliorating SS. The inventor tested this hypothesis in two different spontaneous models of SS, which occur in mice with different genetic backgrounds. NOD.B10-H2^b mice were derived from type 1 diabetes-prone nonobese diabetic (NOD) mice by replacing their MHC I-Ag⁷ loci with the MHC I-A^b from C57BL/10 mice (45). NOD.B10-H2^b mice spontaneously develop exocrine inflammatory infiltrates, anti-nuclear autoantibodies and develop SS with a female predilection (45-48). They do not develop insulinitis, hyperglycemia, or T1D, which could be potential confounds in these studies (49). Exocrine tissue from female NOD.B10-H2^b mice displays robust lymphocytic infiltrates in the salivary and lacrimal glands with progressive loss of saliva and tear secretion. The infiltrates become evident by 3 months in age, and clinical disease manifests at 24 weeks in age with measurable loss of saliva and tear production (45-48).

The inventor also studied C57BL/6.NOD-Aec1Aec2 mice, which are C57BL/6 mice which carry two loci, Idd3 and Idd5 (Aec1 and Aec2), from NOD mice that are necessary and sufficient to cause a SS-like disease and are in synteny with genetic regions associated with SS in humans (50, 51). A systems biology comparison of

genome-wide expression data from the salivary glands of Sjögren's syndrome patients versus that of C57BL/6.NOD-Aec1Aec2 mice identified common dysregulated biological pathways, supporting the notion that these mice provide a good model for studies of interventional therapies (52). In these mice, leukocyte infiltration into exocrine glands begins at 8-16 weeks in age, along with an increase in salivary gland proinflammatory cytokines (13, 16, 50, 51). The infiltrates have a similar composition to that in the human disease, with a similar dramatic increase in Th17 cells. Notably, the elimination of IL-17 in these mice reduces sialadenitis (16). Moreover, transcriptome analysis of their salivary and lacrimal glands at an early stage of the disease revealed the induction of IFN γ -stimulated genes (53). After 16 weeks in age, these mice display pronounced salivary and lacrimal secretory dysfunctions.

Prior to Applicants' invention, there was no effective way to ameliorate or treat SS. Applicants' claimed invention uses

In an embodiment, provided is a method for ameliorating Sjogren's Syndrome, comprising administering to a patient in need thereof an effective amount of one or more GABA-receptor agonists.

In another embodiment, the GABA-receptor agonist is GABA.

In certain embodiments, GABA is administered in an amount of 0.001 gram/day to 30 grams/day, preferably 0.1 gram/day to 10 grams/day.

In another embodiment, an effective amount of an anti-inflammatory compound and/or a corticosteroid is also administered.

In certain embodiments, said GABA-receptor agonist is selected from the group consisting of: GABA_A-receptor agonists, GABA_B-receptor agonists, GABA_A-rho receptor agonists, and combinations thereof.

In certain embodiments, the GABA_A-receptor agonist is selected from the group consisting of: α 5lA, adiplon, beta-alanine, bretazenil, CL-218,872, (-)-epigallocatechin-3-gallate, GABA, gaboxadol, homotaurine, imidazenil, isoguvacine, L-838,417, muscimol, piperidine-4-sulfonic acid, progabide, QH-ii-066, SL-651,498, taurine, zolpidem, and 3-acyl-4-quinolones.

In certain embodiments, the GABA_B-receptor agonist is selected from the group consisting of: baclofen, CGP-44532, GABA, gamma-hydroxybutyrate, isovaline, lesogaberan, phenibut, 3-aminopropylphosphinic acid, and 3-aminopropyl(methyl)phosphinic acid (SKF-97541).

In certain embodiments, the GABA_{A-rho} receptor agonist is selected from the group consisting of: CACA, CAMP, and GABOB.

In certain embodiments, said administration occurs intradermally, intramuscularly, intraperitoneally, intravenously, orally, subcutaneously, sublingually, via aerosol delivery, or via a combination of delivery routes.

In certain embodiments, said administration occurs after the development of sialadenitis in the patient but before the onset of overt symptoms of Sjogren's Syndrome.

In certain embodiments, said administration occurs after the onset of overt symptoms of Sjogren's Syndrome in the patient.

In certain embodiments, prior to the administration step, the method further comprises determining that the patient is afflicted with Sjogren's Syndrome.

In an embodiment, said administration is oral.

By ameliorate is meant to make better or improve, including minimizing or treating the symptoms of SS.

By GABA-receptor agonist is meant an agonist of GABA_A-receptors, GABA_B-

receptors, and/or GABA_{A-rho} receptors (formerly known as GABA_C-receptors).

GABA_A-receptor agonists include: α 51A, adiplon, beta-alanine, bretazenil, CL-218,872, (-)-epigallocatechin-3-gallate, GABA, gaboxadol, homotaurine, imidazenil, isoguvacine, L-838,417, muscimol, piperidine-4-sulfonic acid, progabide, QH-ii-066, SL-651,498, taurine, zolpidem, and 3-acyl-4-quinolones.

GABA_B-receptor agonists include: baclofen, CGP-44532, GABA, gamma-hydroxybutyrate, isovaline, lesogaberan, phenibut, 3-aminopropylphosphinic acid, and 3-aminopropyl(methyl)phosphinic acid (SKF-97541).

GABA_{A-rho} receptor agonists include: CACA, CAMP, and GABOB.

Administration of the GABA-receptor agonist can be intradermally, intramuscularly, intraperitoneally, intravenously, orally, subcutaneously, sublingually, via aerosol delivery, or via a combination of delivery routes. Preferred routes include orally, sublingually, and/or via aerosol delivery.

In some embodiments, the GABA-receptor agonist is/are administered in an amount of 1 ng/kg/day to 500 mg/kg/day. In particular examples, the GABA-receptor agonist is/are administered in amount of 1 ng/kg/day-500 mg/kg/day, 10 ng/kg/day-500 mg/kg/day, 50 ng/kg/day-500 mg/kg/day, 100 ng/kg/day-500 mg/kg/day, 200 ng/kg/day-500 mg/kg/day, 400 ng/kg/day-250 mg/kg/day, 750 ng/kg/day-100 mg/kg/day, 1-1000 μ g/kg/day 50-1500 μ g/kg/day, 100-1000 μ g/kg/day, 150-500 μ g/kg/day, or 200-400 μ g/kg/day.

EXAMPLES

The results of the inventor's studies of prophylactic and interventive GABA-receptor agonist treatment on exocrine functions in the NOD.B10-H2^b and C57BL/6.NOD-Aec1Aec2 mouse models of SS are presented herein.

Materials and Methods

Mice

Female NOD.B10-H2^b mice (the Jackson Laboratory) and C57BL/6.NOD-Aec1Aec2 mice were studied due to their greater propensity to develop sialadenitis. The derivation of C57BL/6.NOD-Aec1Aec2 mice has been previously described (66). Mice were bred and maintained under specific pathogen-free conditions with a 12-hour light/dark cycle in the Division of Laboratory Animal Medicine at UCLA. They were provided food and water *ad libitum*. This study was carried out in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocols for all experiments using vertebrate animals were approved by the Animal Research Committee at UCLA (protocol #1993-2001).

Reagents

GABA, pilocarpine, ketamine, xylazine, and isoproterenol were purchased from Sigma-Aldrich (St. Louis, MO, USA).

GABA Treatment

Mice were randomized and given plain water or water containing GABA (at 6 or 20 mg/ml as indicated) continuously through the drinking water. The drinking water was changed every 5 days. Previous studies have shown that mice given GABA through their drinking water consume the same amount of food and water as mice on plain water (23, 40).

Measurements of Saliva Production

Saliva production was measured as described (16) with slight modifications.

Individual mice were weighed and lightly anesthetized by ketamine (8 microgram/gram of body weight)/ xylazine (4 μ g/gr of body weight). After 5 min, tear and saliva secretion was stimulated by intraperitoneal injection with PBS containing freshly prepared pilocarpine (40 μ g/100 μ l) and isoproterenol (20 μ g /100 μ l). Immediately after stimulation, the secreted saliva was collected with a micropipette that was inserted into the oral cavity. The time to the start of saliva flow was recorded and saliva was collected for 10 minutes thereafter. The collected saliva from each mouse was transferred into a pre-weighed Eppendorf tube and the weight of the secreted saliva was determined. The saliva flow rate was calculated as the weight of saliva collected per gram weight of the mouse.

Measurement of Basal Tear Production

Under light anesthesia, a phenol red thread (ZoneQuick, Ophthalmics, Pembroke, MA) was held in the lateral canthus of each eye with a forceps. After 20 seconds, the thread was removed and the entire wet (red) portion was measured and recorded in millimeters using the scale provided in the Zone-Quick test kit. This was repeated for the other eye and these two measurements were averaged for each mouse.

Histological Analysis of Salivary and Lacrimal Gland Tissues

The day after collecting tear and saliva, the mice were humanely euthanized and their submandibular gland (SMG) and exorbital lacrimal gland (ELG) were excised, fixed in 10% formalin for 24 hours and paraffin-embedded. The tissue sections (4 μ M) were routine-stained with by H&E. Using a Nikon Eclipse 90i upright microscope fitted with a Nikon DS-Qi1Mc camera, the H&E-stained sections were

photoimaged with a 4X objective lens. The number of lymphocytic infiltrate foci (defined as an aggregate of >50 monocytes) were counted in each SMG or ELG section. To determine the infiltrate area the infiltrate areas and the total tissue section area of each image were measured using Nikon NIS-Element software. The data are presented as the mean percentage of infiltrate area in the SMG or ELG sections. One SMG or ELG tissue section was analyzed per mouse. All slides were coded with ID numbers and the histological assessments were performed in a blinded manner.

Statistical Analysis

Data are the mean $\pm$ SD of each group from at least three separate experiments. The difference between groups was analyzed by Student's T-test or Mann-Whitney U test where applicable using online tools. A two-tailed p-value of <0.05 was considered statistically significant.

Example 1 – Prophylactic GABA Treatment in NOD.B10-H2^b Mice

In NOD.B10-H2^b mice, sialadenitis becomes histologically observable beginning at about 12 weeks in age and overt clinical symptoms such as reduced saliva and tear production are apparent by 24 weeks in age (45-48). Eighteen weeks old female NOD.B10-H2^b mice were randomized to continuously receive plain water or water supplemented with GABA (6 mg/ml). Fourteen weeks later, at 32 weeks in age, we analyzed their saliva flow and tear production.

Saliva and tear production was stimulated by isoproterenol and pilocarpine administration as per (54). The inventor observed that GABA-treated NOD.B10-H2^b mice produced an average of 41% more saliva than the NOD.B10-H2^b mice given plain water, although this was not statistically significant (Fig. 1A). The time to saliva

production was significantly shorter in GABA-treated mice ($p < 0.05$, Fig. 1B). Impressively, the amount of tear production was on average about 10-fold higher in the GABA-treated group vs. the plain water-treated group ($p < 0.01$, Fig. 1C). Thus, prophylactic GABA treatment tended to preserve saliva production, significantly shortened the time to saliva production, and greatly increased the amount of tear production relative to the control mice when examined about 8 weeks after the usual time of disease onset.

The next day, the salivary and lachrymal glands of the mice were harvested, processed for H&E staining, and the number of lymphocytic foci in the submandibular gland (SMG) and exorbital lacrimal gland (ELG) of each group were determined. The mean number of lymphocytic foci in the SMG of control and GABA-treated mice were essentially the same (Figs 1D). Similarly, the number of lymphocytic foci in the ELG of control and GABA-treated mice were very similar (Fig. 1F). The area of the infiltration was larger in the SMG and ELG of GABA-treated mice relative to the controls (Fig. 1E and 1G), but these differences did not reach statistical significance.

Example 2 – Interventive GABA Therapy After Disease Onset In NOD.B10-H2^b mice

GABA treatment was initiated at 24-weeks in age, after the appearance of overt clinical symptoms. Twenty-four-week-old female mice were randomized to continuously receive plain water or water containing GABA (20 mg/ml). At 30 weeks in age, the saliva flow and tear production of the mice were analyzed. GABA-treated NOD.B10-H2^b mice produced on an average of 72% more saliva than those given plain water ($p < 0.001$, Fig. 2A). The time to saliva production was also significantly shorter in GABA-treated mice ($p < 0.01$, Fig. 2B). The amount of tear production was

on an average about 4-fold higher in the GABA-treated group vs. the plain water treated group ($p < 0.001$, Fig. 2C). Thus, GABA treatment after the clinical onset of the disease helped preserve saliva and tear production, the most salient symptoms of SS.

The next day, the salivary and lachrymal glands were harvested, processed for H&E staining, and the mean number of lymphocytic foci and the area of infiltrates in each group was determined. Some differences in the number of infiltrate foci and their area in GABA-treated vs. control mice were observed, none of these differences were statistically significant. (Fig. 2). Thus, the GABA-mediated preservation of exocrine function was not associated with significant changes in lymphocytic foci number or area in the SMG or ELG.

Example 3 – Prophylactic GABA Treatment in C57BL/6.NOD-Aec1Aec2 Mice

To further test whether GABA has therapeutic potential for SS treatment, the inventor performed similar studies in the C57BL/6.NOD-Aec1Aec2 mouse model of SS. In these mice, infiltrates become evident in exocrine glands beginning around 8 weeks of age and these mice display pronounced salivary and lacrimal secretory dysfunctions by 16 weeks in age (13, 16, 50, 51, 53).

To test GABA as a prophylactic therapy, the inventor randomized 9 weeks old female C57BL/6.NOD-Aec1Aec2 mice to receive plain water or GABA (20 mg/ml) continuously for 7 weeks. At 16 weeks in age, the mean saliva production in GABA treated mice was 46% greater than that in plain water treated mice, although this was not statistically significant (Fig. 4A), and there was essentially no difference in the time to saliva production between these groups (Fig. 4B). The volume of tears secreted by the GABA-treated mice was 38% greater than that of plain water-treated

mice, although this too was not statistically significant (Fig. 4C). Finally, there were no significant differences in the number of SMG and ELG lymphocytic foci between experimental and control groups (Figs. 4D and 4E).

Example 4 – GABA Treatment After Disease Onset in C57BL/6.NOD-Aec1Aec2 Mice

In a second study with C57BL/6.NOD-Aec1Aec2 mice, the mice were randomized to receive plain water or constant GABA treatment beginning at 16 weeks of age, after the onset of overt disease. These mice were subsequently examined at 20 weeks in age. The inventor found that C57BL/6.NOD-Aec1Aec2 mice which received GABA mice produced an average of 88% more saliva than the control mice given plain water ($p < 0.01$), Fig. 5A). Their time to saliva production was also significantly shorter in GABA-treated C57BL/6.NOD-Aec1Aec2 mice ($p < 0.01$) Fig. 5B). GABA treatment also led to an average of 133% more tear production ($p < 0.01$, Fig. 5C). Paralleling the inventor's observations in NOD.B10-H2^b mice, the inventor observed no significant difference in the number or area of lymphocytic foci in the SMGs or ELGs of GABA treated vs. plain water-treated C57BL/6.NOD-Aec1Aec2 mice (Group data in Figs 6A-D), and representative images in Figs 4C and 4D).

Example 5 – Withdrawing GABA After Certain Benefits of Treatment Are Realized

This study seeks to determine whether the beneficial effects of GABA treatment will continue if the treatment is withdrawn after several weeks on the GABA treatment.

NOD.B10-H2^b mice will be placed on plain water (control group, N = 12) or GABA (treatment group, N = 36) at 24 weeks of age, as described in Examples 1 and 2. After both 2 weeks and 4 weeks of treatment, 12 mice in the treatment group will have their GABA treatment withdrawn and will then be maintained on plain water. This will create groups of mice (N = 12) treated for 0, 2, 4, and 6 weeks with GABA. At 30 weeks in age, certain data will be obtained as described in Examples 1-4, namely: saliva production, time to saliva production, tear production, foci number, and area of infiltration in the SMG and ELG of mice that received GABA for 0, 2, 4, or 6 weeks.

The inventor expects the results of this study to demonstrate that the positive effects of GABA treatment on saliva production, time to saliva production, and tear production will persist, at least partially, for some period of time after the treatment is withdrawn.

Example 6 – Determining Whether GABA Treatment Can Be An Effective Treatment When Treatment Is Initiated Long After Disease Onset

This study seeks to determine whether, after the long-term establishment of Sjogren's Syndrome, the anti-inflammatory actions of GABA treatment can still lead to improvement in salivary and/or ELG exocrine functions.

NOD.B10 mice will be housed as described in Examples 1 and 2 and provided plain water for approximately 30 weeks or 36 weeks of age, at which point some of the mice will be given water with GABA (20 mg/ml, continuously), as described in Examples 1 and 2. This will create the following groups: no GABA administration (control group, N = 12), GABA administration starting at ~30 weeks of age (N = 12), and GABA administration starting at ~36 weeks of age (N = 12). The weight and the food and water consumption of all mice will be assessed weekly.

Every 4 weeks for 3-4 months the saliva and tear production of the mice will be assessed. The precise time of starting and ending treatment will depend on the results.

Given the ability of GABA treatment at the onset of overt disease to preserve saliva and tear production (see, e.g., Fig. 1), the inventor expects that GABA treatment will also have beneficial effects when treatment is initiated well-after disease establishment.

Example 7 – Effects Of GABA In A Humanized Mouse Model of Sjogren's Syndrome

To further confirm the relevance of the mouse model studies to treatment in human patients, the inventor will utilize a chimeric human-mouse model of SS (67). This model involves the adoptive transfer of PBMC from an SS patient to immune-deficient NSG mice (Jackson Laboratory). Previous studies demonstrated that, four weeks after receiving PBMC from SS patients the recipient, mice display a mean 64% reduction in saliva production relative to NSG mice that received PBMC from healthy individuals (67). Additionally, such mice were found to display infiltrates in their SMG and ELG whose composition is largely within the pathological spectrum of the observed in SS patients (67), while NSG mice that received PBMC from healthy donors did not display infiltrates in their SMGs or ELGs (67).

PBMCs will be obtained from patients with primary SS because patients with secondary SS (i.e., SS associated with another rheumatic disease such as rheumatoid arthritis or lupus) may have complex immune and other abnormalities which might complicate interpretation of data. Fresh blood samples will be collected from patients with primary SS and from patients not afflicted with SS (control). PBMC will be isolated from those blood samples using routine techniques.

Each patient's isolated PBMCs will be injected into four NSG mice, with each mouse receiving approximately 5×10^6 human PMBC intraperitoneally (67). Following PBMC injection, two of the mice will receive plain water, and the other two will receive GABA (20 mg/mL) in their drinking water. Four weeks after transfusion, saliva production, time to saliva production, and tear production will be measured. For each PBMC donor, we will calculate the average saliva and tear production in mice treated with GABA vs. plain water treatment.

Based on GABA's ability to reduce inflammatory responses by human PBMC (26, 68), we expect that GABA treatment will be found to preserve saliva and tear production in human-mouse chimeras.

Example 8 – Treatment of SS Using Other GABA-Receptor Agonists

Examples 1-7 will be repeated with other GABA-receptor agonists, such as homotaurine. It is expected that the other GABA-receptor agonists will provide similar therapeutic benefits as were seen with GABA.

Discussion

SS treatment usually involves a combination of symptom management strategies which may be needed life-long. The inventor studied GABA treatment in two different SS mouse models which occur in different genetic backgrounds; NOD.B10-H2^b and C57BL/6.NOD-Aec1Aec2 mice. These mice were given GABA prophylactically or after the appearance of overt symptoms. Weeks after initiating treatment, mice that received GABA treatment prophylactically had greater production of saliva and tears, although the higher levels were not always statistically significant relative to control mice that received plain water. When GABA treatment

was initiated after the appearance of SS symptoms, the beneficial effects of GABA-treatment on exocrine functions were more evident, leading to significantly greater saliva flow and shorter time to saliva flow, as well as more tear flow in both SS models. Thus, the inventor's observations from two different models of mouse SS demonstrate that GABA treatment after the onset of overt disease ameliorates the key symptoms of SS.

Clinical studies with immunosuppressants such as Rituximab, Belimumab, and Abatacept failed to reach the primary endpoints of efficacy in SS patients. These drugs target a specific immune cell population and may not have been effective because by the time SS becomes manifest there may be multiple immune cell populations involved in disease pathogenesis and targeting only one of these populations is insufficient to halt disease progression. Alternatively, these treatments may also target regulatory cells, such as regulatory B and T cells, and reduce their inhibitory activities leading to disease progression. As described above, GABA has a broad range of anti-inflammatory actions, including inhibiting Th17, Th1, and CD8⁺ T cell responses, modulating APC toward more anti-inflammatory phenotypes, and enhancing Tregs, all of which may have contributed to its beneficial effects.

Histologically, the inventor did not observe that the changes in the number and area of SMG or ELG lymphocytic foci were correlated with the improved exocrine functions in GABA-treated mice. Such observations are reminiscent of the past study of IL-27 treatment in C57BL/6.NOD-Aec1Aec2 mice, which also led to improved saliva flow rates without significant changes in SMG lymphocytic infiltrate numbers irrespective of the age at which treatment of the mice was initiated (54). Indeed, although lymphocytic infiltrates in exocrine glands are important criteria for

clinical disease, the extent of these infiltrations often do not correlate with disease severity in SS patients (55, 56). Interestingly, analysis of biopsied salivary glands from SS patients found that Th1 and Th17 infiltrates were largely outside the germinal centers, while Th2 and Tfh cells were localized within the germinal centers. (57). Studies have shown that GABA-R agonists inhibit Th1 and Th17 cells, but not Th2 cells (29, 36, 38), which might explain the persistence of large areas of lymphocytic infiltrates despite the much-improved saliva and tear production in GABA-treated mice. Additionally, studies of NOD mice noted that the number of ELG lymphocytic foci decreased and became more condensed with disease progression (58). Accordingly, a GABA-mediated delay in disease progression may lead to an increase in the number and area of lymphocytic foci. Finally, in the T1D field, some experimental immunotherapies do not reduce the quantity of islet infiltrates, but rather shift their composition toward a “benign insulinitis.” The benign insulinitis is composed of more regulatory cells, such as Tregs, and fewer pro-inflammatory cells and is associated with long-term tolerance to the insulin-producing β -cells (e.g., (59-61)). In a similar fashion, GABA treatment may have led to a more “benign sialadenitis” in which pathogenic cells gave way to regulatory cells. It is also possible that GABA’s ability to inhibit inflammation may have allowed homeostatic acinar cell replication to gradually increase exocrine function.

Oral GABA treatment has been tested in epilepsy patients for its ability to reduce seizures (62-64). While it lacked clinical benefit, probably because GABA is unable to cross the blood-brain barrier, there were no adverse effects. A recent phase Ib GABA oral dosing study indicated that GABA is safe for consumption at up to 6 grams/day (65). Given the preserved exocrine function observed in GABA-

treated SS mouse models, GABA treatment is a promising new approach to help ameliorate SS.

The examples and embodiments described herein are for illustrative purposes only and in no way limiting. All publications, patents, and patent applications referenced herein are hereby incorporated by reference herein for all purposes.

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What is claimed is:

1. A method for ameliorating Sjogren's Syndrome, comprising administering to a patient in need thereof an effective amount of one or more GABA-receptor agonists.
2. The method of Claim 1, wherein the GABA-receptor agonist is GABA.
3. The method of Claim 2, wherein GABA is administered in an amount of 0.001 gram/day to 30 grams/day, preferably 0.1 gram/day to 10 grams/day.
4. The method of any one of Claims 1-4, wherein an effective amount of an anti-inflammatory compound and/or a corticosteroid is also administered.
5. The method of Claim 1, wherein said GABA-receptor agonist is selected from the group consisting of: GABA_A-receptor agonists, GABA_B-receptor agonists, GABA_{A-rho} receptor agonists, and combinations thereof.
6. The method of Claim 5, wherein the GABA_A-receptor agonist is selected from the group consisting of: α 51A, adiplon, beta-alanine, bretazenil, CL-218,872, (-)-epigallocatechin-3-gallate, GABA, gaboxadol, homotaurine, imidazenil, isoguvacine, L-838,417, muscimol, piperidine-4-sulfonic acid, progabide, QH-ii-066, SL-651,498, taurine, zolpidem, and 3-acyl-4-quinolones.

7. The method of Claim 5, wherein the GABA_B-receptor agonist is selected from the group consisting of: baclofen, CGP-44532, GABA, gamma-hydroxybutyrate, isovaline, lesogaberan, phenibut, 3-aminopropylphosphinic acid, and 3-aminopropyl(methyl)phosphinic acid (SKF-97541).
8. The method of Claim 5, wherein the GABA_{A- ρ} receptor agonist is selected from the group consisting of: CACA, CAMP, and GABOB.
9. The method of any one of Claims 1-8, wherein said administration occurs intradermally, intramuscularly, intraperitoneally, intravenously, orally, subcutaneously, sublingually, via aerosol delivery, or via a combination of delivery routes.
10. The method of any one of Claims 1-9, wherein said administration occurs after the development of sialadenitis in the patient but before the onset of overt symptoms of Sjogren's Syndrome.
11. The method of any one of Claims 1-9, wherein said administration occurs after the onset of overt symptoms of Sjogren's Syndrome in the patient.
12. The method of any one of Claims 1-11, wherein, prior to the administration step, the method further comprises determining that the patient is afflicted with Sjogren's Syndrome.
13. The method of any one of Claims 1-12, wherein said administration is oral.

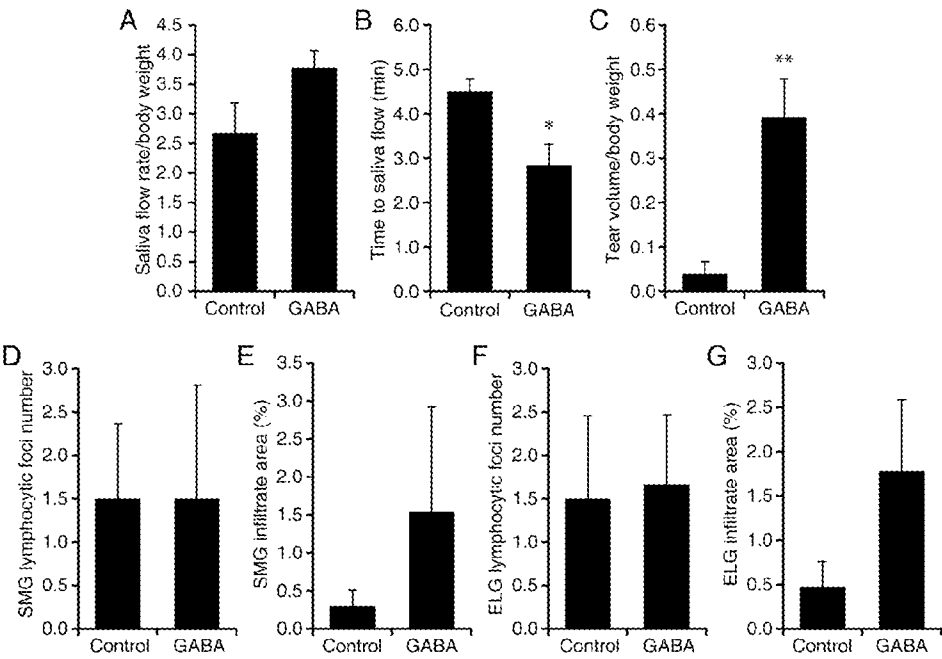


Figure 1

2/6

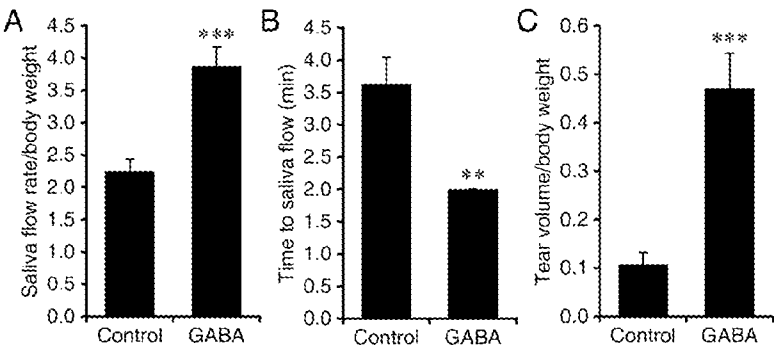


Figure 2

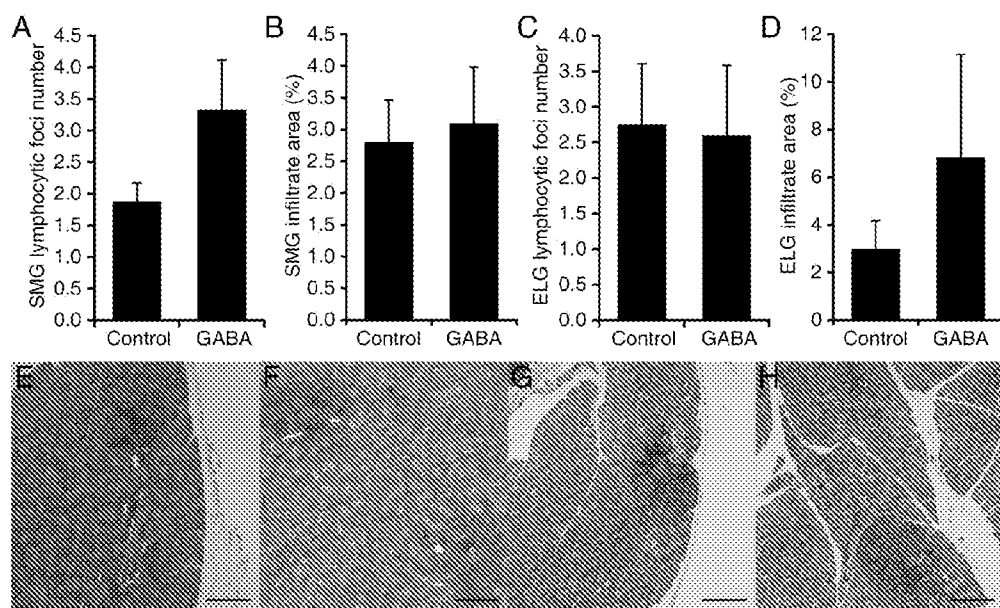


Figure 3

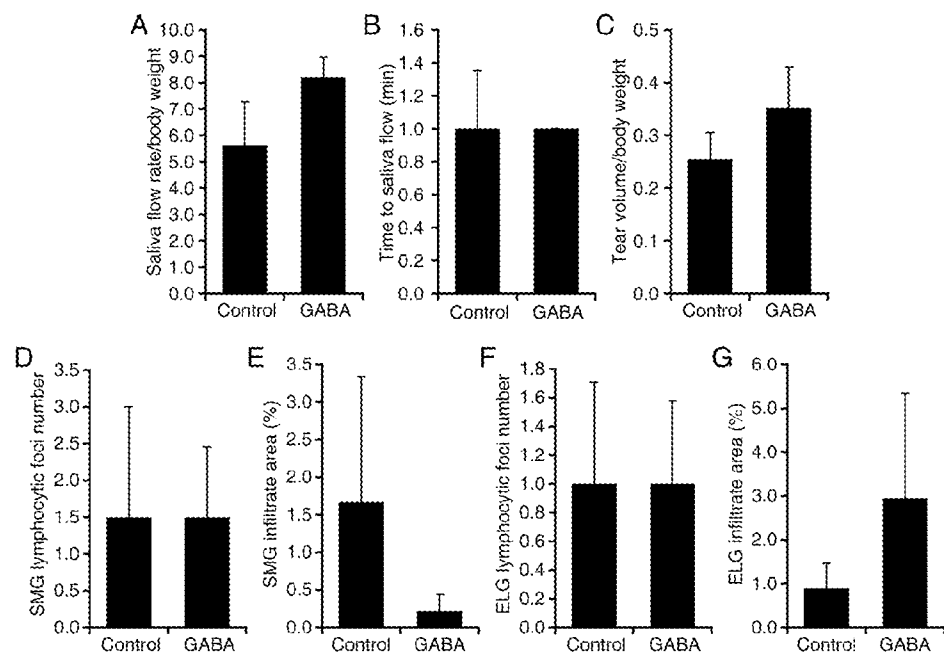


Figure 4

5/6

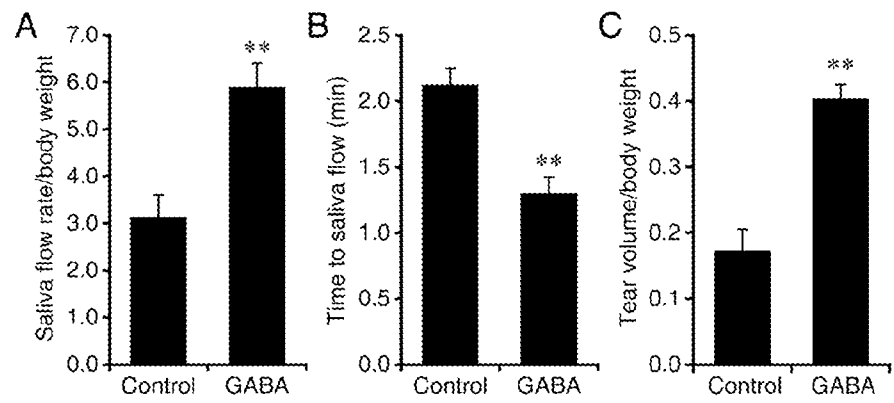


Figure 5

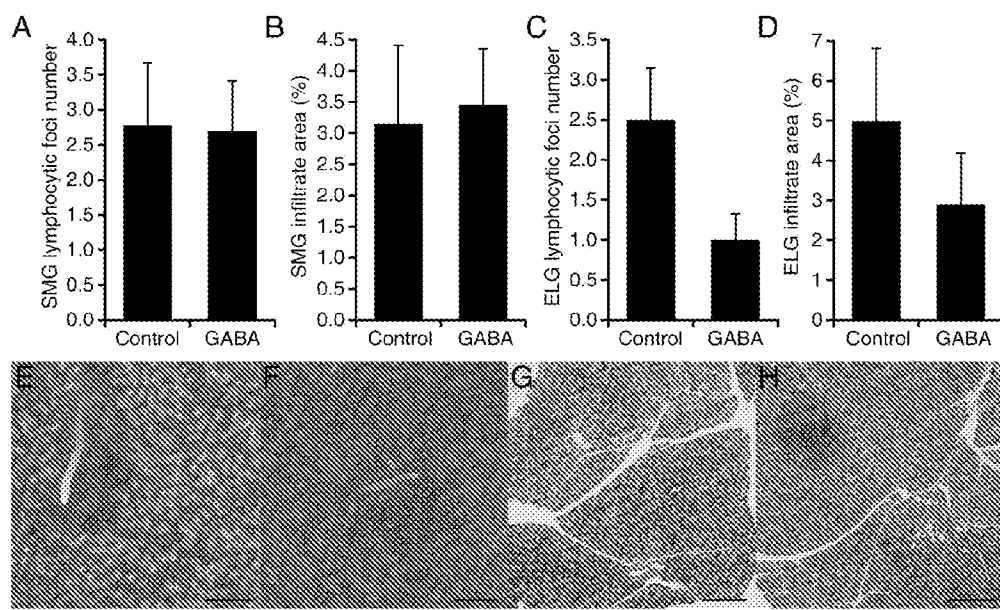


Figure 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/051044

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 9-13
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/051044

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - INV. - A61K 31/131; A61K 31/19; A61P 37/00 (2023.01)

ADD. - A61P 25/00; A61P 29/00 (2023.01)

CPC - INV. - A61K 31/131; A61P 37/00; A61K 31/19 (2023.01)

ADD. - A61P 29/00; A61P 25/00 (2023.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic database consulted during the international search (name of database and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2020/0138830 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA et al) 07 May 2020 (07.05.2020) entire document	1-8
A	WO 2017/058074 A1 (DIAMYD MEDICAL AB) 06 April 2017 (06.04.2017) entire document	1-8
A	WO 2020/191047 A1 (NEUROCYCLE THERAPEUTICS INC) 24 September 2020 (24.09.2020) entire document	1-8
P, X	SONG et al, GABA Administration Ameliorates Sjogren's Syndrome in Two Different Mouse Models, Biomedicines, Vol. 10, Iss. 1, 07 January 2022, Pgs. 1-13	1-8

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

27 January 2023

Date of mailing of the international search report

FEB 22 2023

Name and mailing address of the ISA/

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