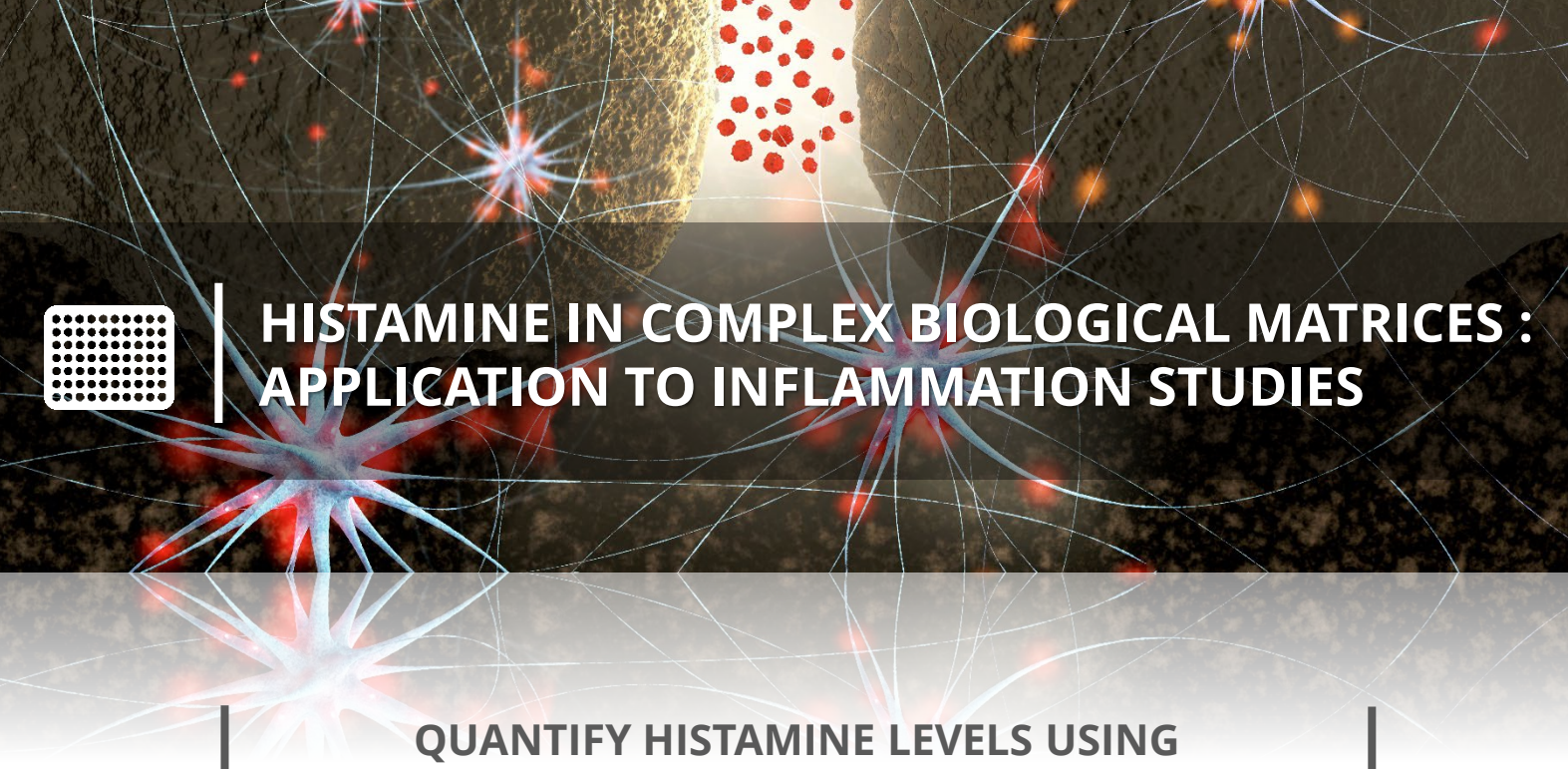

HISTAMINE IN COMPLEX BIOLOGICAL MATRICES : APPLICATION TO INFLAMMATION STUDIES

QUANTIFY HISTAMINE LEVELS USING THE BERTIN BIOREAGENT HISTAMINE ELISA KIT

Histamine is a molecule that plays a major role in local immune responses, is involved in the regulation of the physiological functions of the intestine, and acts as a neurotransmitter for the brain, spinal cord, and uterus. As part of the inflammatory response to a foreign body, histamine is produced and released by basophils and by mast cells. An increase in local histamine levels can lead capillaries to become more permeable to leucocytes and some proteins, enabling them to attack pathogens in infected tissues.

Following its discovery in 1910, histamine has been identified as a central player in the pathophysiology of allergy and asthma, and drugs targeting its receptors have been in clinical use since the 1940s. To understand the role of histamine in inflammatory reactions as well as the immunoregulatory effects of histamine in physiology and various pathologies, it is essential to be able to measure histamine levels accurately. Bertin Bioreagent has designed the Bertin Bioreagent Histamine ELISA kit to detect and quantify histamine in complex biological matrices. The Bertin Bioreagent Histamine kit has been widely cited in inflammation studies.

SUMMARY

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EVALUATION OF HISTAMINE LEVEL IN MOUSE LUNG LAVAGE FLUID

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/ CONTEXT

Histamine is an organic compound that plays a major role in the development of many allergic diseases. Mast cells are multifunctional bone marrow-derived tissue-dwelling cells that are responsible for a major part of histamine production in the body. Most allergic diseases are triggered by the interaction between an allergen and allergen-specific antibodies present on the membrane of mast cells and basophils. This interaction leads to the degranulation of these cells, which in turn provokes the release of many allergic and inflammatory biomarkers, including leukotrienes and histamine. Leukotrienes are inflammatory mediators produced in leukocytes from arachidonic acid by the enzyme 5-lipoxygenase (5-LO). For example in asthma, both the release of cysteinylleukotrienes and the release of histamine from degranulating mast cells are responsible for the narrowing of airways that happens during asthma attacks.

However, currently, there are no approved anti-asthma drugs that can simultaneously inhibit the biosynthesis of leukotrienes – by blocking the activity of the 5-LO enzyme - and mast cell degranulation. The only 5-LO inhibitor approved for the treatment of asthma, Zileuton, has limited efficacy which might be linked to its inability to affect mast cell degranulation [1]. Consequently, drugs that could simultaneously inhibit 5-LO and mast cell degranulation are increasingly being considered as promising therapeutic agents for asthma.

The objective of this study was to evaluate the ability of PH-251 - an oxazolidinone hydroxamic acid derivative known for its ability to strongly inhibit the 5-LO enzyme – to simultaneously inhibit mast cell degranulation. The Bertin Bioreagent Histamine ELISA kit (Bertin Bioreagent, Montigny-le-Bretonneux, France) was used to evaluate the anti-degranulatory effect of PH-251 on *in vivo* lung anaphylaxis (a severe allergic reaction to a chemical). Briefly, the kit was used to measure the histamine content in the lung lavage fluid of mice, as an indicator of mast cell degranulation.

/ MATERIALS

In order to evaluate the anti-degranulatory effect of PH-251 on in vivo lung anaphylaxis, a group of mice was immunized and boosted as described below:

- Animals: BALB/c mice, originally obtained from Harlan Laboratories, Derby, UK, and bred in the facilities of Kuwait University were used. All animals were maintained under temperature-controlled conditions with an artificial 12-h light/dark cycle and allowed standard chow and water ad libitum.
- Immunization, challenge and drug treatment: Mice were immunized intraperitoneally as described in [2] with 10 µg ovalbumin mixed with 0.2 ml of aluminum hydroxide gel (Alu-Gel-S; SERVA Electrophoresis GmbH, Heidelberg, Germany), on days 0 and 7. Mice were separated into 5 treatment groups of 8 animals per group. Seven to ten days after the allergen booster dose, the different groups of animals were pretreated subcutaneously with the drug vehicle or PH-251 (30 mg/kg) or disodium cromoglycate (DSCG) (30 mg/kg) - an anti-asthma drug known to promote mast cell stabilization - or zileuton (30 mg/kg), 1 h before being challenged intra-tracheally with 50 µl of ovalbumin solution (0.25%) or PBS.
- Measurement of histamine content in mouse lung lavage fluid: Thirty minutes after the challenge, the animals were killed and the lungs immediately lavaged with 2 × 0.5 ml PBS. Lavage fluid was immediately centrifuged and the supernatant diluted 1: 10 in 0.1 N $HClO_4$ and stored at − 70 ◦C till assay of histamine content as index of mast cell degranulation. Histamine assay was done using the Bertin Bioreagent Histamine ELISA kit (Bertin Bioreagent, cat # A05890, Montigny-le-Bretonneux, France, previously sold under the brand SPI-BIO). Values were expressed as histamine concentration (ng/ml) in the original lavage fluid and can be seen in Figure 1.

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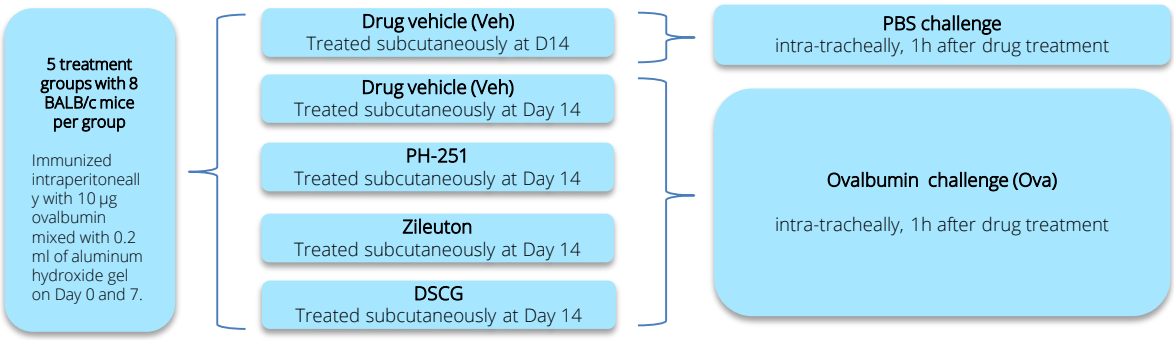
/ STUDY DESIGN



/ RESULTS

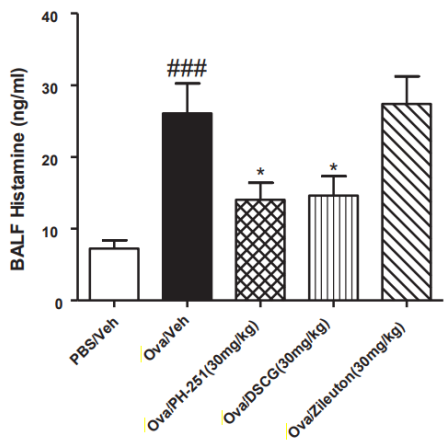


Figure 1: The effect PH-251, disodium cromoglycate (DSCG) or zileuton on the histamine content of the bronchoalveolar lavage fluid (BALF) recovered from various animal groups. Ovalbumin (Ova)-sensitized and boosted animals were pre-treated subcutaneously with the drugs or vehicle 1 h before intra-tracheal challenge with 50 µl of Ova (0.25% solution) or PBS. Thirty minutes after challenge, animals were killed and the lungs lavaged with 2 × 0.5 ml PBS. The histamine content of the supernatant of the lavage fluid was determined by enzyme immunoassay. ###p < 0.001 (with respect to PBS/Veh) and *p < 0.05 (with respect to Ova/Veh). Values are mean ± SEM, n = 6–8 animals. From [2].

/ CUSTOMER

1. Knapp, Howard R. "Reduced allergen-induced nasal congestion and leukotriene synthesis with an orally active 5-lipoxygenase inhibitor." New England Journal of Medicine 323.25 (1990): 1745-1748.
2. Ezeamuzie, Charles I., et al. "Anti-allergic, anti-asthmatic and anti-inflammatory effects of an oxazolidinone hydroxamic acid derivative (PH-251)–A novel dual inhibitor of 5-lipoxygenase and mast cell degranulation." International Immunopharmacology 105 (2022): 108558.

As can be seen in **Figure 1.**, the antigen challenge (with ovalbumin) led to an increase in the histamine concentration of the lung lavage fluid which was significant compared to the PBS-challenged group. The lung lavage fluid of the mice groups that were treated with PH-251 or disodium cromoglycate (DSCG) had a lower histamine concentration compared to the antigen-challenged mice group. In contrast, zileuton, a clinically available anti-asthma drug, had no effect on histamine concentration. These findings can be taken as indirect evidence of the anti-degranulatory effect of PH-251 on in vivo lung anaphylaxis. The Bertin Bioreagent Histamine ELISA kit allows for accurate measurements of histamine content in mouse lung lavage fluid.

BIOR-026-DU017-B

MEASUREMENT OF HISTAMINE LEVEL IN RAT COLON HOMOGENATES

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/ CONTEXT

Irritable bowel syndrome (IBS) is a common condition arising from both genetic and environmental factors. It is characterized by a group of symptoms affecting the digestive system, including abdominal discomfort and altered bowel movements. Little is known about the environmental triggers for IBS. IBS symptoms might result from an altered immune response. [1]. Aluminum is a common additive in food and is also largely present as a food contaminant through contact with kitchen utensils or packaging. In Europe, it was estimated recently that the tolerable ingested amount of aluminum is far exceeded in a large share of the population, especially in children [2]. It has been shown that aluminum ingestion at a dose of 1.5mg.kg.d altered gut homeostasis and the expression of tight junction proteins in epithelial cells [3].

The objective of this study was to investigate the effect of aluminum ingestion in rodent models of visceral hypersensitivity. The Precellys 24 homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) was used to homogenize colon samples of rats that were treated orally with aluminum citrate (AlCi, a dietary form of aluminum). Then the **Bertin Bioreagent Histamine ELISA kit** (Bertin Bioreagent, Montigny-le-Bretonneux, France) was used to evaluate the **histamine levels in rat colon homogenates, as a marker for inflammation.**

/ PROTOCOL

- **Animals:** Adult Sprague Dawley rats (100–150 g) were purchased from Janvier Labs (Le Genest St Isle, France). For the results shown here, only male rats were used.
- **Treatments:** Rats were administered orally with aluminum citrate (AlCi) (a dietary form of aluminum) (Pfaltz & Bauer, Waterbury, CT, ref. A16090) at dosages of 1.5 mg·kg body weight·d for 1 month, as detailed in the figure legends. A dose of 1.5 mg·kg·d, corresponds to the high value of dietary aluminum ingested by humans.
- **Sample preparation:** Colonic tissue samples were homogenized with the Precellys 24 homogenizer (Bertin Technologies, Montigny le Bretonneux, France) and the **CK28 2mL Precellys lysing kit** (2.8mm ceramic beads, Bertin Technologies, Montigny le Bretonneux, France, ref. P000911-LYSK0-A).
- **Histamine measurements:** Histamine levels were detected in colon homogenates according to the manufacturer's instructions with the **Bertin Bioreagent Histamine ELISA kit** (Bertin Bioreagent, Montigny-le-Bretonneux, France, ref. A05890.96). Readings from tissue samples were normalized to total protein content as detected by DC protein assays (Bio-Rad, ref. 5000111). Results can be seen in **Figure 1.**
- **Statistics:** Data are expressed as mean ± SD. Differences between groups were compared using the Mann-Whitney nonparametric U test (GraphPad Prism version 5.03, GraphPad Software, La Jolla, CA) (*P < .05 in the figures).
- **Study approval:** The animal treatment protocol was approved by the regional bioethics committee (committee no.75; authorization no.CEEA2016030317128286, May 23, 2016) and all of the animals received human care in accordance with European guidelines (Directive 86/609/EEC, European Economic Community, November 24, 1986).

/ RESULTS

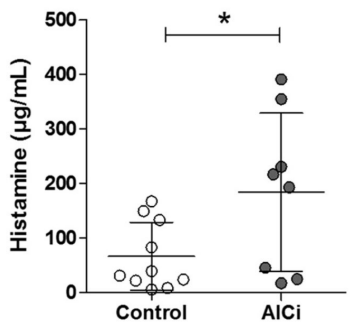

Figure 1. A dose of 1.5 mg·kg·d aluminum induced low-grade inflammation in the colon. Colon histamine levels were analyzed in the colons of rats administered orally with water (Control) or 1.5 mg·kg·d AlCi for 1 month. Colon histamine levels determined by enzyme-linked immunosorbent assay kits (n = 8–10/group). *P < .05, using the Mann-Whitney nonparametric U test. Adapted from [4].

/ CUSTOMER

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1. Öhman, Lena, Hans Törnblom, and Magnus Simren. "Crosstalk at the mucosal border: importance of the gut microenvironment in IBS." Nature reviews Gastroenterology & hepatology 12.1 (2015): 36-49.
2. Arnich, Nathalie, et al. "Dietary exposure to trace elements and health risk assessment in the 2nd French Total Diet Study." Food and Chemical Toxicology 50.7 (2012): 2432-2449.
3. de Chambrun, Guillaume Pineton, et al. "Aluminum Enhances Inflammation and Decreases Healing in Experimental Models of Colitis." Gastroenterology 5.140 (2011): S-493.
4. Esquerre, Nicolas, et al. "Aluminum ingestion promotes colorectal hypersensitivity in rodents." Cellular and Molecular Gastroenterology and Hepatology 7.1 (2019): 185-196.

The results in Figure 1. show that aluminum citrate ingestion at a dose of 1.5 mg·kg·d induces an increase in rat colon histamine contents, which indicates mast cell activation by aluminum. These findings suggest that aluminum is a key player in mast cell–mediated hypersensitivity in rats treated orally with aluminum citrate at dosages relevant to human exposure. Aluminum could be the first identified dietary risk factor for Irritable Bowel Syndrome. The Bertin Bioreagent Histamine ELISA kit allows for accurate measurements of histamine content in rat colon homogenates.

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EVALUATION OF HISTAMINE CONCENTRATIONS IN HUMAN BASOPHIL CULTURE SUPERNATANTS

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/ CONTEXT

Intravenous immunoglobulin (IVIG) is commonly used to treat auto-immune diseases and systemic inflammatory diseases. IVIG contains normal IgG molecules and is prepared from the pooled plasmas of healthy donors. High doses of IVIG therapy have been shown to have anti-inflammatory effects [1, 2].

Basophils are a type of white blood cells that are responsible for inflammatory reactions during the immune response, and also produce inflammatory mediators such as histamine and serotonin. Recently, it has been reported that, in autoimmune and systemic inflammatory disease models, the anti-inflammatory effects of IVIG are mediated through basophils [3].

The objective of this study was to evaluate the effect of IVIG on human basophil functions. Briefly, circulating basophils were isolated from healthy donors and cultured in the presence of several cytokines and IVIG. The effects of IVIG were evaluated based on several inflammatory biomarkers, including histamine release. The effects of IVIG were first examined on resting basophils, but no effects were found, which indicates that resting basophils are not the target for IVIG. Then, the effects of IVIG were investigated on basophils that were stimulated with IL-3 (interleukine-3), the major basophil-priming cytokine [4]. **The Bertin Bioreagent Histamine ELISA kit** (Bertin Bioreagent, Montigny-le-Bretonneux, France) was used to **measure histamine levels in culture supernatants of IL-3-primed human basophils.**

/ PROTOCOL

- **Isolation and culture of basophils**: Basophils were isolated from PBMCs of healthy donors' buffy bags (Centre Necker-Cabanel, EFS, Paris; INSERM-EFS ethical permission nos. 12/EFS/079 and 18/EFS/033) by using the Basophil Isolation Kit II (Miltenyi Biotec, Bergisch Gladbach, Germany) and autoMACS (Miltenyi Biotec). The purity of basophils based on expression of FcεRI and CD123 was approximately 97%. Cells ($0.1x10^6$ cells/well per 200 mL) were cultured in 96-well U-bottomed plate either alone in serum-free X-VIVO 15 medium, with IL-3 (100 ng/mL; ImmunoTools, Friesoythe, Germany), or with IL-3 plus IVIG (25 mg/mL) or human serum albumin (HSA; 10 mg/mL; LFB, Les Ulis, France) or $F(ab')_2$ fragments (16 mg/mL) or Fc fragments (9 mg/mL) for 24 hours to investigate the effect of IVIG on IL-3–primed basophils. Also, basophils were sequentially stimulated with IL-3 and IL-33 for 1 hour each and cultured with IVIG or HSA for an additional 22 hours.
- **Measurement of histamine levels:** Histamine levels were measured in culture supernatants by using the Histamine EIA Kit (Bertin Bioreagent, Montigny-le-Bretonneux, France). Results can be found in **Figure 1**.
- **Statistical analysis:** Statistical analysis was performed with Prism 6 software (GraphPad Software, La Jolla, Calif). One-way ANOVA (with Tukey multiple comparison tests or Dunnett multiple comparison tests), and 2-way Mann-Whitney tests were used to determine statistical significance.

/ RESULTS

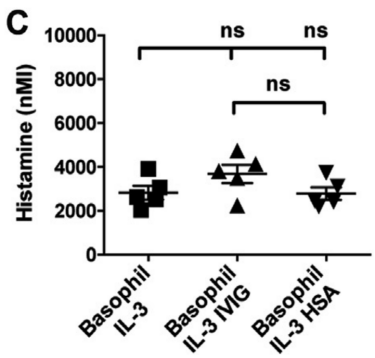

Figure 1: Activation of IL-3–primed basophils by IVIG is not associated with degranulation. Amount of histamine in culture supernatants (means 6 SEMs, n 5 5 donors). ns, Not significant, 1-way ANOVA with Tukey multiple comparison tests. Adapted from [4].

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In this work, the modulating effect of IVIG on IL-3-primed human basophils was examined. IL-3 is considered to be the major basophil-priming cytokine. IVIG were not found to significantly alter histamine release in the culture supernatants of IL-3-primed human basophils even if other inflammatory biomarkers were affected. These results shed new light on the mechanisms of activation of human basophils by IVIG and the differences between human subjects and mice. The Bertin Bioreagent Histamine ELISA kit (Bertin Bioreagent, Montigny-le-Bretonneux, France) can enable researchers to quantify histamine concentrations accurately in culture supernatants.

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SELECTED PUBLICATIONS ON HISTAMINE QUANTIFICATION WITH THE BERTIN BIOREAGENT HISTAMINE ELISA KIT

/ SIALYLATION OF IMMUNOGLOBULIN E IS A DETERMINANT OF ALLERGIC PATHOGENICITY

Immunoglobulin E (IgE) antibodies are major players in the pathophysiology of allergies. Little is known as to why total and allergen-specific IgE concentrations do not reproducibly correlate with allergic disease. It has been shown that glycosylation patterns of IgG (immunoglobulin G) affect its function and are subject to change depending on the pathological state. But the role and effect of glycosylation for IgE remain poorly understood. In this study, an unbiased analysis of glycosylation patterns of IgE was analyzed in two populations of mice: individuals with a peanut allergy and on-atopic individuals without allergies. The **Bertin Bioreagent Histamine kit** (Bertin Bioreagent, Montigny-le-Bretonneux, France, formerly sold under the brand SPI-BIO) **was used to quantify histamine levels in mouse serum samples.** Results show that IgE glycosylation, and specifically sialylation, plays a major role in the regulation of allergic reactions.

https://www.nature.com/articles/s41586-020-2311-z

Shade, Kai-Ting C., et al. "Sialylation of immunoglobulin E is a determinant of allergic pathogenicity." *Nature* 582.7811 (2020): 265-270.

/ OMEPRAZOLE INHIBITS IGE-MEDIATED MAST CELL ACTIVATION AND ALLERGIC INFLAMMATION INDUCED BY INGESTED ALLERGEN IN MICE

Eosinophilic esophagitis is an allergic condition that affects the esophagus. This condition has been characterized by an increased number of mucosal mast cells in patients. The drug omeprazole – a well-known proton pump inhibitor– has been shown to lower esophageal mast cell counts and reduce inflammation in patients previously diagnosed with Eosinophilic esophagitis. The objective of this study was to examine the effects of omeprazole on mast cell functions. Several inflammation biomarkers were measured in the culture supernatant of activated murine bone marrow-derived mast cells, and the effect of omeprazole on these biomarkers was evaluated. The **Bertin Bioreagent Histamine ELISA kit** (Bertin Bioreagent, Montigny-le-Bretonneux, France, formerly sold under the brand SPI-BIO) **was used to evaluate the release of histamine in the culture supernatant of activated murine bone marrow-derived mast cells**. Results show that histamine release -which can serve as an indicator for mast cell degranulation- was reduced by omeprazole in a dose-dependent way.

https://www.sciencedirect.com/science/article/pii/S0091674920303420?casa_token=QwP96MKgf8oAAAAA:hxW7b3wQGaGy65fCermo6dyBqe6ApOGpKsR56_RbJWVBpvpCrR7xgkr1AYVKw2nAY9UilPvPpA

Kanagaratham, Cynthia, et al. "Omeprazole inhibits IgE-mediated mast cell activation and allergic inflammation induced by ingested allergen in mice." *Journal of Allergy and Clinical Immunology* 146.4 (2020): 884-893.

/ HISTAMINE H2 RECEPTOR NEGATIVELY REGULATES OLIGODENDROCYTE DIFFERENTIATION IN NEONATAL HYPOXIC-ISCHEMIC WHITE MATTER INJURY

Neonatal hypoxic-ischemic encephalopathy (HIE) is a disorder that affects between 0.1 and 0.8% of newborns and that can lead to cerebral palsy and lifelong neurological damage. HIE has been characterized by white matter injury, with demyelination and lower numbers of differentiated oligodendrocytes. Demyelinated lesions can be rescued by the recruitment and subsequent differentiation of oligodendrocytes which eventually leads to remyelination. However, the remyelination process fails in newborns that have been diagnosed with HIE. In the case of neonatal HIE, a recruitment of oligodendrocytes precursor cells has been observed in demyelinated areas, but their differentiation into mature oligodendrocytes seems to be hindered. For this reason, it has been hypothesized that the promotion of the differentiation of oligodendrocytes could rescue demyelinated regions and improve clinical outcomes in HIE. However, very few therapeutic targets for the promotion of oligodendrocyte differentiation have been identified. In this study, the regulatory effect of the histamine 2 receptor (H2R) on oligodendrocyte differentiation is examined. Briefly, histamine levels in the corpus callosum of a group of mice were evaluated at several time points after neonatal HIE and compared to a control group, using the **Bertin Bioreagent Histamine ELISA kit** (Bertin Bioreagent, Montigny-le-Bretonneux, France, formerly sold under the brand Bertin Pharma). Results indicate that the histamine 2 receptor (H2R) in oligodendrocytes could be a promising new therapeutic target to treat neonatal HIE.

https://rupress.org/jem/article-pdf/218/1/e20191365/1050276/jem_20191365.pdf

Jiang, Lei, et al. "Histamine H2 receptor negatively regulates oligodendrocyte differentiation in neonatal hypoxic-ischemic white matter injury." *Journal of Experimental Medicine* 218.1 (2021).

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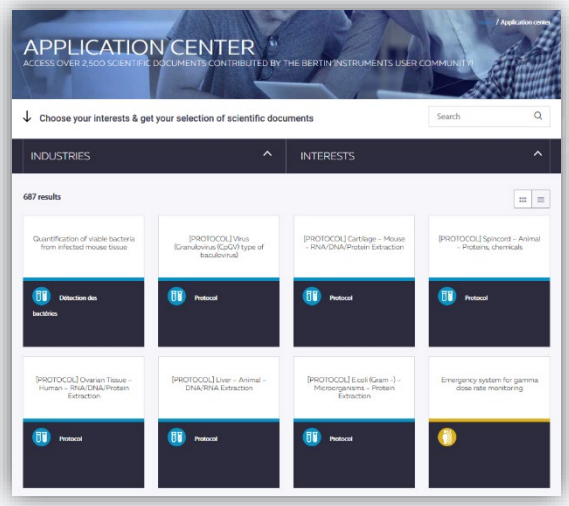

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