UNIVERSITAT ROVIRA i VIRGILI

EFFECTS OF COMMON DRUGS ON DIAMINE OXIDASE ENZYME ACTIVITY AND THE CORRELATION BETWEEN HISTAMINOSIS AND GASTROINTESTINAL SYMPTOMS IN HUMANS

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BIOCHEMISTRY AND MOLECULAR BIOLOGY

FINAL PROJECT DEGREE

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Tarragona, June 2021

ACKNOWLEDGEMENTS

I would like to thank you all those people who have been part of this stage and have helped in every possible way in the realisation of this project.

I would especially like to thank my family, principally my mother, my father and my sister, who have always been there for me in good times and bad. For convincing me day by day that I can achieve my goals and know how to get the best out of me.

To my tutor Ximena Terra Barbadora, for her time, dedication and trust. For helping me to carry out my idea and guiding me through the process of this research.

To my lifelong friends, for their interest, support and company. For having a shoulder to laugh on but also to cry on.

To my friendships I have made over the last four years, for making them unforgettable and above all for always making me feel comfortable and welcomed.

And to my partner, for giving me strength and encouraging me at all times. Thank you for your patience and for being present every day.

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ABSTRACT

The human gastrointestinal system is complex. During the journey from the upper and lower gastrointestinal tract, digestive enzymes play a fundamental role and their correct functioning depends, among others, on good external circumstances as they can be easily affected by environmental conditions such as diet or drugs.

In addition, the gastrointestinal tract possesses an innate and adaptive mucosal immune system that can give rise to the well-known food allergic reactions. However, in contrast to these immunological reactions, food intolerances, are caused by various components (dairy products; sulfite- and salicylate-containing products; fermentable oligosaccharides, disaccharides, monosaccharides and polyols; biogenic amines; gluten; lactose; and food additives).

The project focuses on the biogenic amine histamine. In humans, histamine can be degraded either by the enzyme histamine-N-methyltransferase or by diamine oxidase. The second one, which is mainly found in the intestinal mucosa, degrades exogenous histamine to metabolites that are excreted in the urine. A malfunction of this enzyme leads to histamine intolerance, i.e. an increase in the concentration of histamine in the organism and, consequently, to symptoms in the respiratory, cardiovascular, epithelial, central nervous, digestive, muscular and even skeletal systems. Deficiency of diamine oxidase enzyme activity may be due to genetic, pathological or pharmacological causes. The focal point of the study is the latter, with the aim of finding out why and how various common drugs affect the correct activity of this enzyme. It shows the reversible effect of certain drugs on the enzyme, inhibiting it through interactions with its active site. The relationship with nowadays common gastrointestinal symptoms and the importance that should be given when diagnosing and treating patients are also discussed.

Key words: gastrointestinal tract, biogenic amines, histamine intolerance, DAO, DAO deficiency, DAO-inhibiting drugs.

RESUMEN

El Sistema digestivo humano es complejo. Durante el trayecto desde el tracto gastrointestinal superior e inferior tienen un papel fundamental las enzimas digestivas cuyo correcto funcionamiento depende de unas buenas circunstancias externas ya que pueden verse fácilmente afectadas por condiciones ambientales como son la dieta o los fármacos.

Además, el tracto gastrointestinal posee un sistema inmunitario innato y adaptativo en las mucosas que puede dar lugar a las conocidas reacciones alérgicas alimentarias. Sin embargo, en contraposición a estas reacciones inmunológicas encontramos las intolerancias alimentarias, causadas por diversos componentes (productos lácteos; productos que contienen sulfitos y salicilatos; oligosacáridos, disacáridos, monosacáridos y polioles fermentables; aminas biógenas; gluten; lactosa; y aditivos alimentarios).

El trabajo presente se centra en la amina biógena histamina. En humanos, la histamina puede ser degradada por la enzima histamina-N-metiltransferasa o por la diamino oxidasa. Ésta última, que se encuentra principalmente en la mucosa intestinal, degrada la histamina exógena dando lugar a metabolitos que se excretan a través de la orina. El mal funcionamiento de esta enzima conlleva a sufrir intolerancia a histamina, un aumento de la concentración de histamina en el organismo y, por consiguiente, a la aparición de síntomas en los sistemas respiratorio, cardiovascular, epitelial, nervioso central, digestivo, muscular e incluso sistema óseo. El déficit de actividad enzimática de la diamino oxidasa puede deberse a causas genéticas, patológicas o farmacológicas. El estudio hace énfasis en esta última pretendiendo conocer cuáles son las causas por las que diversos fármacos comunes pueden afectar a la correcta actividad de esta enzima. Se muestra la afectación de ciertos fármacos sobre la enzima de manera reversible, inhibiéndola a través de interacciones con su centro activo. Se comenta también la relación con los síntomas gastrointestinales comunes en la actualidad y la importancia que debe darse a la hora de diagnosticar y tratar a pacientes.

Palabras clave: tracto gastrointestinal, aminas biógenas, intolerancia a histamina, DAO, deficiencia de DAO, fármacos inhibidores de DAO.

ABBREVIATIONS

ACV	acyclovir
ALDH	aldehyde dehydrogenase
BA(s)	biogenic amine(s)
CA	clavulanic acid
CCK	cholecystokinin
CNS	central nervous system
CQ	chloroquine
CTC	chlortetracycline
DAO	diamine oxidase
DDO	D-aspartate oxidase
EECs	enteroendocrine cells
FAD	flavin adenine dinucleotide
FD	functional dyspepsia
FODMAPs	fermentable oligosaccharides, disaccharides, monosaccharides and polyols
GI	gastrointestinal
GLP-1	glucagon-like peptide
HIT	histamine intolerance
HNMT	histamine-N-methyltransferase
IBS	irritable bowel syndrome
ICU	intensive care units
K_i	dissociation constant
K_m	Michaelis-Menten constant
MAO	monoamine oxidase
SIBO	small intestinal bacterial overgrowth
TMQ	trimethoxybenzylaminoquinazoline

1. INTRODUCTION

1.1. DIGESTIVE SYSTEM AND DIGESTIVE ENZYMES

Digestive enzymes, produced by the gastrointestinal (GI) system, are those that degrade carbohydrates, fats and also proteins. The GI system secretes these enzymes to fulfil macromolecular digestion including nutrients assimilation. (1) The gastrointestinal tract of vertebrates and invertebrates characteristics of morphology and function, regularly reveal food chemistry, like macronutrients content and materials refractory to rapid digestion. With relatively modest excess capacity, the expression of digestive enzymes and nutrient transporters approximately matches the dietary load of their respective substrates.

Natural toxins can affect key features such as digestive transit, enzymatic degradation, microbial fermentation, and absorption in foods. (2)

The digestive system in mammals is constituted by glandular organs involving different classes of cells specialized in the secretion of enzymes. In spite of the existence of this cells, others can be associated with the glandular organs or cells and also the intestinal ones to support the digestion; for example neurons, smooth muscle cells and enteroendocrine cells (EECs). The purpose is to carry out extracellular digestion of, as it has been mentioned, food particles. To complement this action, organs such as the small intestine has soak up capacity. (3)

1.1.1. Human Gastrointestinal tract: anatomy, secretion and absorption

In the GI tract of humans it can be two differentiated zones: the upper GI tract and the lower GI tract which are formed of various different organs. Particularly, the upper GI tract is composed by the mouth, esophagus, stomach duodenum, jejunum, and ileum whereas the lower GI tract concerns to the colon, rectum, and anus. (4)

Ingested food is propelled down the esophagus from the pharynx to the stomach by a series of esophageal peristaltic movements. When the food bolus reaches the stomach, it is mixed with gastric acid and digestive enzymes. As a result, it is broken down, allowing the digested material, now called chyme, to pass through the pyloric sphincter and into the duodenum. Once in the small intestine (duodenum, jejunum and ileum), where the digestive process mainly takes place but also the absorption of food and electrolytes, proteins, fats and carbohydrates are broken down by the action of digestive enzymes into smaller units that will be suitable for nutrients absorption. In addition to these organs, there are other so-called accessory organs (the salivary glands, pancreas, liver and gall bladder) that assist in the digestion process. Finally, when the luminal contents reach the large intestine, leftover material that is not absorbed from the small intestine passes through the ileocaecal valve into the colon where the mucosa of the large intestine is responsible for absorption of water, solidification of the contents of the colon into faeces and its storage that will later be expelled through the rectum and anus. (4)

The gastrointestinal tract has a functional anatomy that allows it to perform digestion in a coordinated manner. This is because the anatomy is made up of several layers, most notably an inner mucosal layer arranged by absorptive and secretory epithelial cells. In addition to this, the GI tract layers are joined by a nerve layer, the submucosal layer, which has lymphatic vessels and connective tissue; a smooth muscle layer composed, as its name suggests, of smooth muscle; and an outer serous layer. Specialised EECs that are dispersed and diffusely located in the gastrointestinal mucosa are capable of sensing luminal contents and secreting signalling molecules. These signalling molecules pass into the circulation acting as classical hormones and

acting locally and paracrine on neighbouring cells and also on various neural pathways, both enteric and extrinsic. Each distinct type of EEC has a characteristic distribution along the GI tract. In terms of mediators released, the main ones are cholecystokinin (CCK) and glucagon-like peptide (GLP-1) which play an important role in the reflex control of GI function and in the regulation of food intake. (4)

The epithelial cells of the GI tract are important as they are relevant for the secretion and absorption of solutes, fluids and also electrolytes, varying in structure and function depending on their location along the GI tract which is capable of secreting up to 9 L of fluid daily. This secreted fluid contains digestive enzymes, bile, ions, water and mucus.

In addition to epithelial cells, gastric parietal cells in the glands within the gastric body are also important as they are involved in the secretion of gastric acid and intrinsic factor. Pepsinogen is secreted by the principal cells within the gastric body and hormones (gastrin, histamine, serotonin and somatostatin) are released by the EECs throughout the stomach. (4)

1.2. ENVIRONMENTAL CONDITIONS AND THE GASTROINTESTINAL TRACT

The gastrointestinal tract possesses an innate and adaptive mucosal immune system. The purpose of this immune barrier is to detect the contents found in the lumen, differentiating between various innocuous antigens, including commensal microbiota and food antigens (oral tolerance), as opposed to toxic pathogens. (4)

In relation to environmental conditions, the gastrointestinal tract plays a special role. This organ system serves as the primary gateway between the organism and the outside world. As a result, environmental factors that harm the gastrointestinal tract not only damage the organ system overall, but also modify the purpose of the portal of entry for most of these factors. By altering the delivery of agents to the organism, factors that damage the gastrointestinal tract modulate their own toxic effects or the effects of other agents on the person. Environmental conditions can reduce or enhance the injury response. (5)

The person at risk is exposed to disease or damage to the gastrointestinal tract. This risk is defined on the basis of age, sex, general health, diet, hepatic smooth endoplasmic reticulum induction status and substance, ethanol, and tobacco use. (5)

1.2.1. Adaptation of digestive enzymes to the diet

Clearly, any change in protein, carbohydrate, or lipid consumption affects the enzymes that hydrolyze these substances. Increased starch intake, for example, boosts pancreatic amylase activity, which leads to more disaccharides being generated. The activity of the enzyme disaccharidase has been shown to rise as a result of the above increase. This is appropriate for the digestion of proteins and lipids as well. (6)

Under the influence of pancreatic and intestinal enzymes, dietary components are hydrolyzed primarily in the intestinal part of the digestive tract. (6) As it can be observed in Figure 1, polypeptides, oligopeptides and amino acids are able to stimulate the activation and the secretion of pancreatic enzymes such as trypsin, chymotrypsin, elastase and carboxypeptidase.

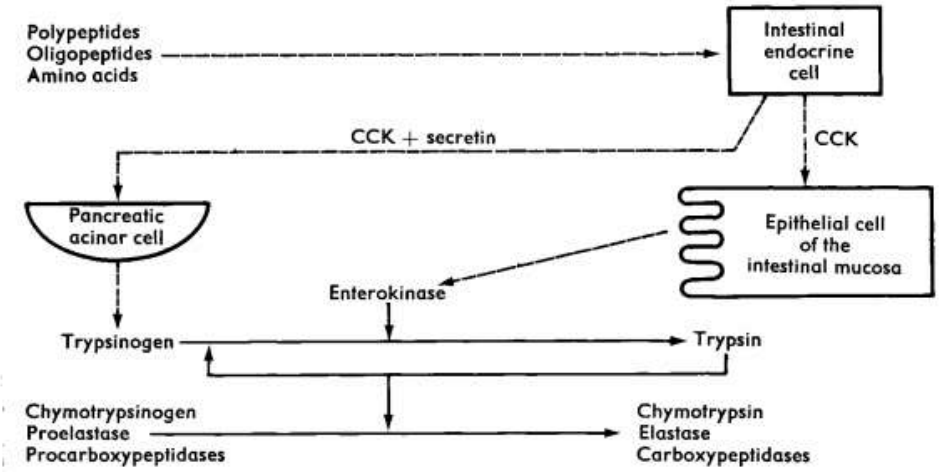

Figure 1. Pancreatic enzyme secretion and activation (Freeman, H.J., Kim, Y.S., 1978).

1.2.1.1. Protein digestion: a complex process

Undegraded dietary proteins and polypeptides from gastric digestion are hydrolyzed concurrently or sequentially by pancreatic proteolytic enzymes and intestinal peptidases once they reach the duodenal lumen. According to some studies, digestive enzymes adjust mainly to the amount of nitrogenous material consumed. (5)

Pancreatic proteolysis releases amino acids and peptides into the intestinal lumen. Amino acids, as well as certain peptides containing glycine or proline, are absorbed. Peptidase enzymes, found at the brush border of the intestinal mucosa, and intracellular peptidases, found in the cytoplasm of enterocytes, are involved in the hydrolysis and absorption of other peptides. Brush boundary enzymes are not the same as intracellular enzymes, according to research. (5)

Diet has one of the early effects on the increase of these enzymes, implying that they are adaptive. The amount of protein consumed have a stimulating effect on peptidase activity, indicating that it is a unique adaptive process rather than the translation of a general enzymatic reaction. (5)

One of the putative functions of cytoplasmic peptidases could be the hydrolysis of absorbed peptides; the substrate choice is critical in determining enzyme activity. However, cytoplasmic peptidases with similar electrophoretic profiles to intestinal peptidases can be found in a variety of organs. (5)

Because of their lack of organ specificity, these intracellular enzymes may have a broader role in intracellular protein metabolism rather than a particular role in peptide digestion. Eventually, absorption mechanisms are affected by the amount of protein consumed. (6)

1.2.1.2. Carbohydrate digestion: the process of breaking down carbohydrates

The primary dietary carbohydrate, starch, is hydrolyzed into maltose, triose, and dextrins in the intestinal lumen by pancreatic amylase. Disaccharidases are enzymes that break down disaccharides into simple sugars. The intracellular glycolytic enzyme team in some species guarantees fructose to glucose conversion. (5)

In the digestion of this macromolecules any change in the amount of starch consumed has a big impact on pancreatic amylase levels. Diet affects the activity of amylase in pancreatic juice. (5)

Sucrose, maltose, and lactose are the most common disaccharides in human and animal nutrition. In reference to the intestine, disaccharidases must knock them down into monosaccharide components before they can be absorbed (sucrase, maltase and lactase). (5)

The brush border of the intestinal mucosa has been identified as a source of these enzymes, and several human and rat studies have found that some disaccharidase activities differ depending on the type and amount of carbohydrate substrate.

Sucrose and maltose administration therefore increases specific sucrase and maltase activities, whose adaptation does not appear to be specific because all disaccharidase activity increases regardless of the carbohydrate consumed. (6)

1.2.1.3. Digestion of lipids

In the presence of bile salts and colipase (lipase cofactor with pancreatic origin) pancreatic lipase hydrolyzes dietary lipids, which are mainly triglycerides, in the intestinal lumen.

Bile micelles transport the released monoglycerides and fatty acids to the mucosa of the intestine, where they are absorbed. Enzymatic re-esterification takes place in the enterocyte. (5)

The pancreas is in charge of enzyme secretion to degrade the dietary lipids. Pancreatic lipase adaptation to dietary lipids has been observed and validated in rats, as well as dogs, chickens, and pigs. The type of lipids used in the various diets may have a different effect; unsaturated fatty acids induce lipase synthesis more than saturated fatty acids. (5)

A really significant increases in specific lipase activity could however be partly attributed to a very high protein, carbohydrate-free diet. In context of assimilable animal energy, the ratio of carbohydrate to lipids in the diet would explain why variations in lipase activity are non-existent or high. (5)

The extracted fatty acids and monoglycerides are mostly absorbed in the jejunum following intestinal hydrolysis. Changes in the amount of lipid substrate digested cause the intestinal re-esterification process to react. Monoglyceride absorption rises in tandem with the adaptive increase in monoglyceride transferase activity. (6)

Natural foods have different effects than semi-synthetic diets, and even how food is cooked has an impact. However, in addition to diet, as mentioned above, digestive enzymes are affected by other environmental factors. Individual factors that are not genetically determined play a large role in drug and chemical metabolism. (5)

1.2.2. Adaptation of digestive enzymes to drugs

Diet is a factor that can alter metabolism and thus have an impact on enzyme secretion. However, it is not the only factor with the capacity to modulate the homeostasis of the body.

Hepatic microsomal enzymes are triggered by many drugs and chemicals. The alimentary tract, especially the small intestine, is also stimulated to produce such enzymes. Enzymes in the gastrointestinal tract are caused by environmental chemicals, altering their own and other absorption of agents. (5)

Drugs are administered to patients in order to reduce or eliminate the symptoms being caused by a disease or pathology. Therefore, once the drug has entered the body, it interacts with

specific receptors or enzymes, called molecular targets, on which it exerts its effects, both therapeutic and adverse. These molecular targets are responsible for propagating the drug signal through effector pathways. The process leads to the effects of the drug, which are usually seen or felt, as the drug is designed with the intention of interfering with the disease or eliminating symptoms. (7)

For a large number of drugs, the therapeutic effect is mediated by a known target protein. However, undesirable adverse effects may occur through the same mechanism of action as the therapeutic effect or through different mechanisms. This is how drugs can modulate enzymes, as they will either be their molecular targets or they may have an undesirable effect on them in an undesirable way, affecting, among other things, to endogenous and exogenous substrates as shown in Figure 2. (7) It is assumed that digestive enzymes are included in the term enzymes.

	Endogenous substrates	Exogenous substrates
Enzyme inhibition	· Deficiency of essential metabolite, production of abnormal and biologically inactive metabolites, or accumulation of intermediary products which become toxic when present in excess	· Increased levels of parent drug resulting in dose-dependent toxicity · Decreased bioinactivation of an active metabolite resulting in idiosyncratic toxicity
Enzyme induction	· Increased metabolism of an endogenous substrate resulting in a deficiency state	· Increased production of a toxic metabolite resulting in either dose-dependent (idiosyncratic) toxicity

Figure 2. Adverse drug reaction effects: variation of enzyme activity with respect to endogenous and exogenous substrates. (Pirmohamed M, Park BK, 1999)

1.3. FOOD ALLERGY AND INTOLERANCE

Flatulence, indigestion, and allergic reactions to a normal diet are all too common in our daily lives. Specific foods and food items could cause a wide range of reactions, from mild to life-threatening anaphylaxis. These adverse reactions are divided into immune-mediated reactions, such as food allergies, and non-immune-mediated conditions, such as food intolerance or sensitivity. (9)

When a person has a food allergy, the immune system mistakenly identifies those food proteins as allergens, triggering an immune reaction that results in a variety of allergies. Food intolerance, on the other hand, is a non-immune mediated adverse reaction. Food allergy could be classified into three types: enzymatic, pharmacological, and unknown or idiopathic food allergy, and it can occur right away or take up to 48 hours. (9)

Food aversion or allergy may appear as a variety of symptoms, including pruritus, swelling, tingling, and angioedema on the skin; bronchoconstriction, dyspnoea, and wheezing on the respiratory system; and stomach cramps and nausea on the gastrointestinal system. It is worth noting that allergic and intolerance reactions differ depending on the individual, the region, the weather, and other factors. (9)

Dairy products; sulfite- and salicylate-containing products; fermentable oligosaccharides, disaccharides, monosaccharides and polyols, which are short-chain carbohydrates (FODMAPs); biogenic amines; gluten; lactose; and food additives are among the most common food intolerances. (9)

This review focuses on biogenic amines (BAs) intolerance, on the basis that BAs or biologically active amines are low molecular weight organic bases that can be found in a wide variety of foods. (9)

1.3.1. Food intolerance: Biogenic amines (BAs)

BAs are nitrogenous compounds found in fermented foods or foods exposed to microbial contamination, they are formed by the transformation of food aminoacids by microorganism enzymatic action. (10)

They are essential metabolites of dietary protein and amino acids that can also be generated in the gastrointestinal tract by gut digestive enzymes and microbes. They play a critical role in the regulation of intestinal functions such as digestion, absorption, and local immunity. Nevertheless, high concentrations of them can cause adverse reactions in animals and can be detrimental to their health, so they could pose a health risk to consumers. (11)

1.3.1.1. Synthesis and occurrence in food

BAs are generated by microbial enzymes decarboxylating particular amino acids, producing CO_2 and amines in the process. (7) It is important to note that BAs formation is influenced by the bacterial strain(s) present, the level of decarboxylase activity, and the accessibility of the amino acid substrate. (12) They can be formed exogenously and be eaten or they can be formed endogenously. In both ways, once they reached the GI system, they are metabolized and are capable to interact with functional signalling pathways, illustrated in Figure 3.

Histamine, tyramine, cadaverine, tryptamine, 2-phenylethylamine, putrescine, agmatine, spermidine, and spermine are the most important relative precursors and BAs contained in the food. Among them, one of the most important BAs is histamine. (7)

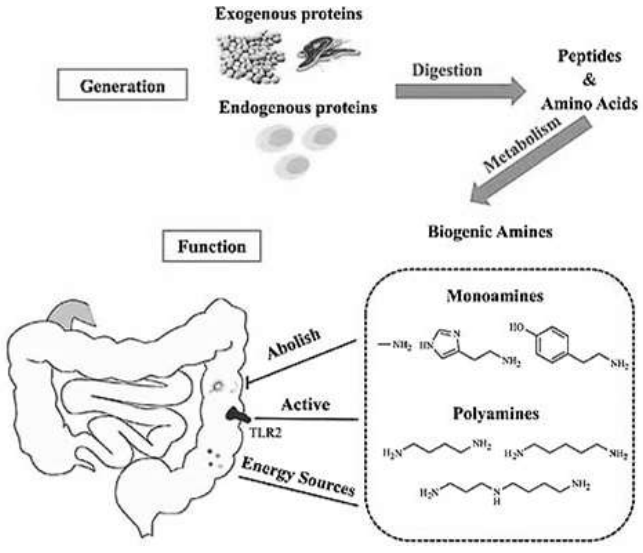

Figure 3. Interaction of different biogenic amines with the functional signalling pathways in the intestinal body and monitorization of the content of biogenic amines in the gastrointestinal tract. (Muthukumar J. et al., 2020).

Some fruits as tomatoes or banana, spinach, chocolate, peanuts, crustaceans or egg white, also seafood, pork and other additives and spices are examples of plant and animal-derived foods that have natural histamine-releasing potential that increases with maturation. BAs has also been detected in scombroid fish tissues, as well as dried fish, fermented fish, vacuum wrapped fish, and fermented cold-smoked fish. (9)

According to European legislation (EC 2073/2005), histamine levels in fresh fish are limited to 200 mg/kg, and cured fish products are limited to 400 mg/kg. On another note, lactic acid fermented vegetables like carrot and beetroot BAs levels range from 1 to 15 mg/kg. (9)

In addition, BAs are also often present in spoiled foods, with cadaverine and putrescine being the most popular. Tyramine levels in fermented meat products are higher. Histamine, tyramine, and putrescine are all present in trace quantities in sauerkraut, which is a fermented plant-based food. Apart from that, soy products are an excellent source of BAs since most soy-based products are fermented or infected with Bacillus spp., which have high amino acid decarboxylation activity. (9)

In terms of dairy products, the level of BAs varies between milk, yogurt and cheese, having the first one the lower level, increasing respectively. In particular, cheese contains tyramine, histamine, tryptamine, putrescine, and cadaverine but depending on the milk, BAs content can differ as the ones made by raw milk have higher BAs in comparison to those that are pasteurized or produced by ewe and goat milk. (9)

Moreover, in wine there is also a variation in the level of biogenic amines, whereas white wine includes lower level of histamine, red wine have a higher content and that is because they are submitted to a different fermentation process. Furthermore, beer has also higher levels of histamine and tyramine and that has been linked to microbial contamination during the brewing process. Since yeast and microbiota drive the fermentation process, cider, a fermented alcoholic beverage made from apple juice, is a good source of histamine, tyramine, and putrescine. (9)

1.3.1.2. Histamine

In an enzymatic reaction (Figure 4) involving L-histidine decarboxylase (EC 4.1.1.22), the bioactive amine Histamine (2-[4-imidazolyl] ethylamine) is synthesized from its precursor amino acid, histidine. (13)

Histamine is classified as a heterocyclic diamine with an imidazole ring and ethylamine due to its chemical structure and number of functional groups (i.e., a functional group in the form of a primary amine given by an organic compound). (13)

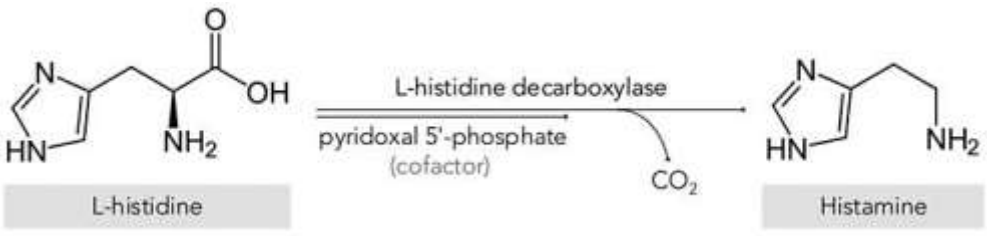

Figure 4.Histamine (BA) synthesis by L-histidine decarboxylase as from descarboxylation of L-histidine (precursor amino acid) with the aid of a cofactor. (Comas-Basté et al., 2020).

This BA is a common constituent of tissues that is formed by all living beings. As a result, humans are the primary source of histamine whereas the derived from food source is the second. (14)

Histamine is produced and stored in large quantities in secretory granules, primarily in basophils and mast cells, but also in gastric enterochromaffin cells, lymph nodes, and the thymus. It stimulates gastric acid secretion, inflammation, smooth muscle cell contraction, vasodilation, and cytokine formation, among other processes, and it is involved in various immune and physiological mechanisms. Histamine is also a neurotransmitter, as it is formed by neurons in the posterior hypothalamus. (10)

What triggers these physiological effects is the interaction between histamine, which acts like ligand, and four G-protein-coupled receptors (H1, H2, H3, and H4), which sets off signal transduction pathways. (10)

The effects of histamine on the cardiovascular system are mediated by both H1 and H2 receptors. The H2 receptor mediates the chronotropic response to histamine in the heart, while the H1 receptor mediates the inotropic impact. H1 receptors are involved in activities including bronchoconstriction, vasoconstriction, and oedema development, all of which can be harmful. The modulation of pulmonary H2 receptors causes bronchodilation and inhibits mediator release. H2 receptors are also found in the brain and the gastric mucosa, explaining the stimulant effect on gastric acid secretion by histamine. (15, 16)

Histamine H3 receptors function as presynaptic autoreceptors in the central nervous system (CNS), inhibiting the production and release of histamine in histaminergic neurons, also in CNS. (16)

The H4 receptor is found in immune system cells and mast cells, and it causes eosinophils and mast cells to chemotaxis. T lymphocytes, dendritic cells, and basophils have all been shown to have it. The H4 receptor, along with the H2 receptor, is thought to play a role in human lymphocyte IL-16 secretion regulation. Moreover, the H4 receptor shares a lot of similarities with the H3 receptor (58% for transmembrane areas, but 34-35% overall), and several H3 agonists and antagonists also bind to the H4 receptor. (16) Figure 5 is a schematic representation of the H1-H4 histamine receptors and their consequent effects of binding to their ligand.

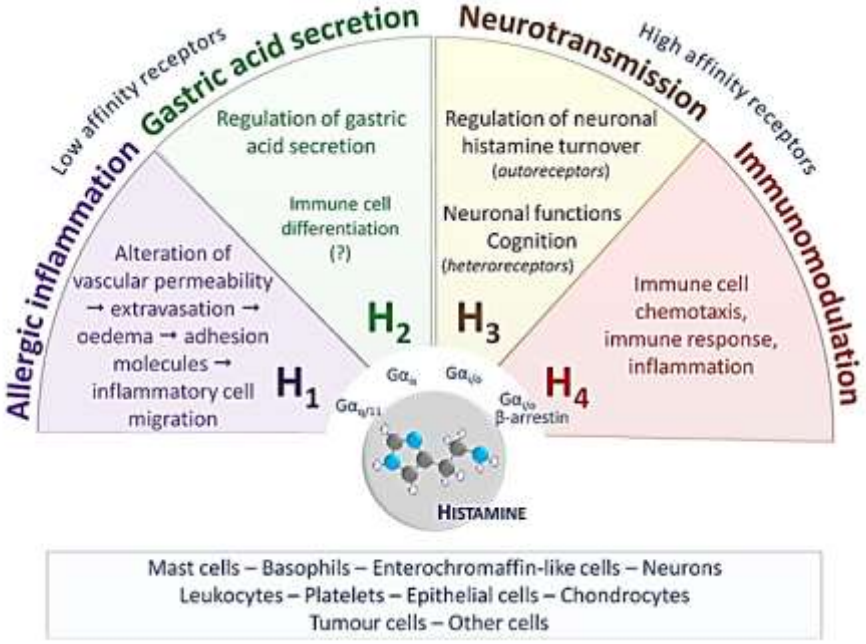

Figure 5. Description of the receptors of histamine key functions. The variety of cell types that produce Histamine are shown in the rectangular box. Gas, Gai/o, Gaq/11: G protein Ga subunits. (Tiligada E, Ennis M, 2020)

In humans, two different principal metabolic pathways exist in relation to histamine (Figure 6). On the one hand, the responsible of one pathway is the enzyme diamine oxidase (DAO) (EC 1.4.3.22), termed as histaminase or amiloride-binding protein too. It is a copper-dependent amino oxidase enzyme encoded by the AOC1 gene located on chromosome 7 (7q34-36). DAO is a homodimer with two isoforms which function is to catalyze the oxidative deamination of the primary amine group of histamine whereas on the other hand, the enzyme histamine-N-methyltransferase (HNMT) (EC 2.1.1.8), a small monomeric protein encoded by a gene located on chromosome 2q22.1, is able to metabolize histamine into 1-methylhistamine. In this case, the purpose for the enzyme is to catalyze the methylation of the secondary amine group of the histamine imidazole aromatic heterocycle. This is possible due to a reaction that requires the S-adenosyl methionine co-substrate as a methyl group donor. (14)

As a result, the enzymes DAO and HNMT deaminate or methylate, respectively, the histamine present in the body, depending on its position. (10) DAO thus achieves inactivation and elimination of extracellular histamine while HNMT mainly degrades histamine in liver tissue. (14)

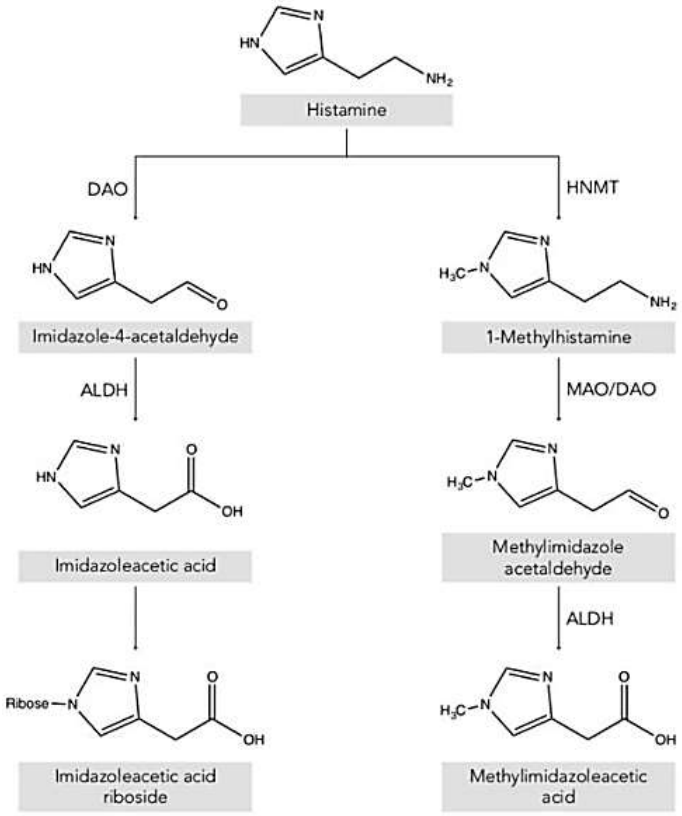

Figure 6. Histamine metabolism in humans: two different signaling pathways including the enzymes DAO: diamine oxidase; HNMT: histamine-N-methyltransferase; ALDH: aldehyde dehydrogenase; MAO: monoamine oxidase. (Comas-Basté et al., 2020).

1.3.1.3. Diamine oxidase (DAO): expression and selectivity

This review is focused specially in the histamine signalling pathway involving the enzyme DAO. To go deeper about this enzyme, it has to be mentioned that it is a secretory protein which is responsible for the degradation of extracellular histamine and it is contained in vesicular structures of the plasma membrane. (12)

The intestinal epithelium serves as a gateway for dietary histamine into the body. While HNMT is found in the gastrointestinal tract, DAO, which is more strongly expressed, protects the body from exogenous histamine, if it comes from ingested food or is generated by the intestinal microbiota. (12)

DAO expression particularly in mammals is only found in a few tissues, primarily the small intestine, ascending colon, placenta, and kidneys. In contrast, HNMT is present in many different areas of the human body including the kidneys and liver, as well as the spleen, intestine, uterus, ovaries, spinal cord cells, and the trachea and respiratory tract. Moreover, DAO activity rises gradually in the intestine from the duodenum to the ileum, and it is found mainly in the intestinal villi. (12)

As far as substrates, there are also some differences between enzyme DAO and HNMT due to the selectivity for histamine. It has been observed that HNMT has a high selective level for this BA. However, although DAO is able to metabolize preferably histamine, it is also capable to metabolize other BAs like putrescine and cadaverine. This explains why Michaelis–Menten enzymatic constant of HNMT (K_m: 6–13 mol/L) is lower than DAO one (K_m: 20 mol/L). (12)

2. OBJECTIVES

Considering the frame of reference, the gastrointestinal system can adapt or undergo variability due to various factors. The revelation of the existence of food intolerance to biogenic amines and the role of the DAO enzyme in the body allows us to focus on histamine intolerance caused by DAO enzyme activity deficiency. In this review there is a special focus on the possible pharmacological effects on this enzyme as well as the interference of several common drugs on its signalling. Therefore, the aim is to review the studies that demonstrate DAO deficiency due to pharmacological causes and to find out what the causes of the interference are.

These objectives have been carried out by a method of bibliographic search in various databases and documents, especially in the National Center for Biotechnology Information (NCBI), mainly PubMed, but also Scopus, the European Bioinformatics Institute of the European Molecular Biology Laboratory (EMBL-EBI) and Google Scholar. The research focused principally on histamine and histamine intolerance, DAO enzyme and inhibition of DAO by drugs among others. A wide variety of articles were achieved and their consequent reading and selection of the most recent literature allowed to accomplish an exhaustive review of the subject presented.

3. HISTAMINE INTOLERANCE INVOLVING DAO REDUCED ACTIVITY

The imbalance between cumulative histamine and histamine degradation induces histamine intolerance. The latter is primarily based on diamine oxidase (DAO) reduced activity, with histamine-N-methyltransferase deficiency playing a smaller position (HNMT). (18)

Diarrhoea, headache, rhinoconjunctivitis, asthma, hypotension, arrhythmia, urticaria, pruritus, and flushing are some of the symptoms and signs that may occur after consuming this biogenic amine, which is found in varying quantities in many foods and alcohol. Histamine intolerance, in addition to being induced by increased consumption of these foods/drinks, can also be caused by the use of some medications, as it has been suggested that certain drugs trigger histamine release or block DAO. (18)

DAO blocks exogenous histamine from passing through the epithelial barrier, so if DAO function is hampered, it results in an increasing enteral histamine absorption, higher plasma histamine levels, and symptoms. After the causes of acquired histamine intolerance have been eliminated, the condition can be intermittent and hence reversible (i.e. discontinuing DAO-blocking drugs). (18)

3.1. GASTROINTESTINAL SYMPTOMS CAUSED BY INHIBITED DAO

Different disorders, such as food allergy and intolerance to sulphites, histamine, or other biogenic amines, tend to have common clinical effects and are triggered by some foods and beverages. The presence of histamine intolerance caused by a deficiency in the function of the enzyme that degrades histamine is frequently underestimated or its symptoms misinterpreted, making differentiation of the causative agent in adverse reactions to food, alcohol, and drugs a difficult task. (19)

Despite the wide variety of symptoms that can occur due to this enzyme dysfunction, one of the most common is migraine, with studies focusing on it while gastrointestinal symptoms are partially neglected. However, in addition to headaches, histamine intolerance can cause gastrointestinal issues like diffuse stomach pain, colic, flatulence, and diarrhoea. Inflammatory and neoplastic disorders including Crohn disease, ulcerative colitis, allergic enteropathy, food allergy, and colorectal cancer have been linked to increased histamine levels and reduced DAO activities. (19)

More than 20% of the population is affected by unexplained, chronic, functional gastrointestinal symptoms. Deficiency of DAO activity leads to histamine intolerance (HIT) resulting in functional, non-specific, non-allergic and even extraintestinal gastrointestinal complaints. (20)

Patients with unexplained gastrointestinal ailments or various related disorders and diseases are usually not evaluated for HIT, i.e. it is not included in the differential diagnoses of such patients. The clinical diagnosis of HIT is still a challenge. Therefore, possible methods of detection are being investigated. It is important to keep in mind that histamine should also be considered in the differential diagnosis of patients with various diseases and disorders of unknown origin but associated with functional gastrointestinal complaints. At the moment it has not been established that values of DAO enzyme in the serum of the patient correlate with DAO activity in the gut. Nevertheless, a low serum DAO value may support the diagnosis of HIT. (20)

Due to the lack of specificity of symptomatology, these symptoms described by patients usually do not lead to a clear diagnosis. With regard to irritable bowel syndrome (IBS), 80% of patients suffering from IBS identified foods that include histamine in their composition as factors causing their symptoms. (21)

In addition to IBS, a number of other pathologies that give rise to similar complaints are now known as IBS-like disorders. Within these disorders we differentiate between functional dyspepsia (FD) and small intestinal bacterial overgrowth (SIBO). The factors that give rise to these pathologies or ailments are still being understood and this is where the role of HIT comes in. With the wide variety of symptoms caused by this intolerance, it could be one of the triggers for these disorders. In fact, a study of etiologic factors has been carried out in which nickel (Ni) plays an important role, as it has been characterised in patients with symptoms similar to those of IBS as a possible causal factor. Interestingly, several of the foods in which Ni has been identified also contain histamine. As a consequence, patients are not able to digest these foods well, which could be attributed to a case of HIT. (21)

What we currently know about the relationship between the intake of certain food groups and the pattern of GI symptoms, psychological symptoms and quality of life is poor. In a study (Böhn L, et al., 2013) with 197 IBS patients (mean age 35 (18-72) years; 142 women), a food questionnaire was used to identify the food groups and specific foods that, according to the testimonies of IBS patients, are responsible for GI-related symptomatology. The association with gastrointestinal and psychological symptoms and the quality of life of these patients was also studied. They were also provided a questionnaire assessing general depression and anxiety

(hospital anxiety and depression), GI-specific anxiety (visceral sensitivity index), IBS symptoms (IBS severity scoring system), somatic symptoms (patient health questionnaire-15) and quality of life (irritable bowel syndrome quality of life questionnaire). (22)

As a result, 84% of the patients stated that at least one of the foods listed in the survey caused their symptoms. 70% of these patients referred to symptoms related to malabsorption of carbohydrate foods (dairy products (49%), beans and/or lentils (36%), apple (28%), flour (24%) and plum (23%)). Of these, about 58%, had gastrointestinal symptoms due to the intake of foods rich in BAs (wine and/or beer (31%), salami (22%) and cheese (20%)). In addition, milk (43%), wine/beer (31%) and pork (21%), which are histamine-releasing foods, were also classified as a cause of symptoms in IBS patients. (22)

However, no association was seen between foods reported as initiators of GI complaints and body mass index, age, IBS subtype, anxiety, depression or GI-specific anxiety. Finally, a large proportion of these foods were linked to reduced quality of life, affecting energy and fitness, sleep and social functioning. Because of this, self-reported food intolerance is associated with a high list of symptoms and reduced quality of life. (22)

As discussed in previous sections, approximately 20% of Europeans regularly take drugs that inhibit the DAO enzyme, which significantly increases their susceptibility to the adverse effects of exogenous histamine like IBS. Several of these DAO-inhibiting drugs, including high-dose acetylsalicylic acid and non-steroidal anti-inflammatory drugs, are available without a prescription. However, they can cause gastrointestinal side effects, including, with prolonged use and at high doses, an increased risk of gastrointestinal bleeding. Therefore, treatments involving long-term use of these drugs need to be considered and evaluated. (20)

3.2. DIAGNOSIS AND TREATMENT OF DAO ACTIVITY DEFICIENCY OR HIT

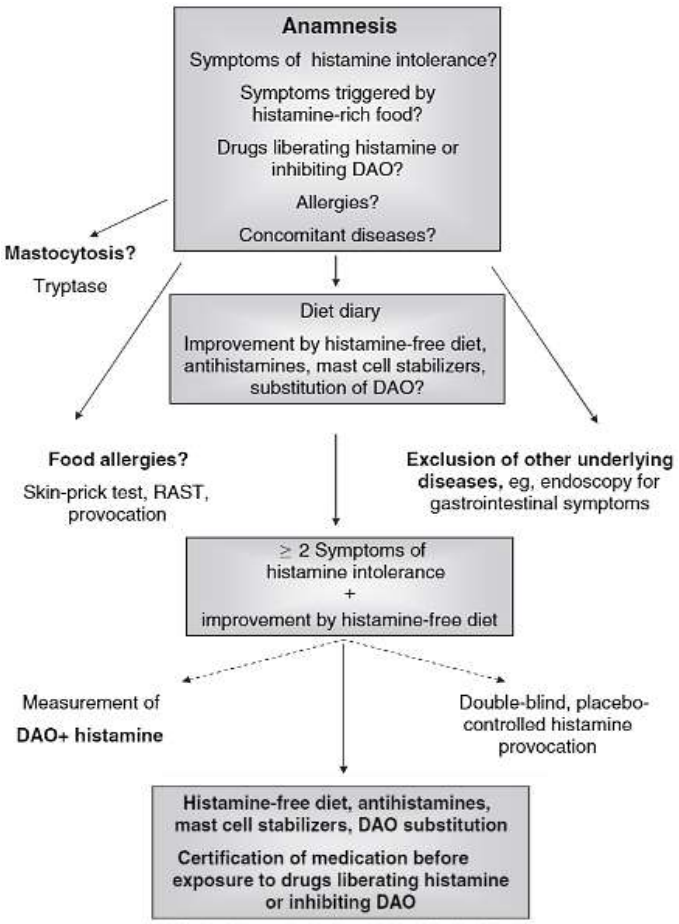

Figure 7. Possible pathway for the diagnosis of HIT. (Maintz L, Novak N, 2007)

Prior to treatment, it is important to detect HIT. Correctly identifying which pathology or disorder a patient suffers from is crucial for recovery.

It has already been mentioned that it has not been established that serum DAO values correlate with DAO activity in the gut, but a low serum DAO value could support the diagnosis of HIT. (20)

In Figure 7 it is shown a suggestion of schematic process about the detection of HIT and possible treatments.

On the other hand, a standardised questionnaire (Figure 8) based on a complete anamnesis of all HIT-related complaints has been proposed, which could be instrumental in diagnosing HIT. (20)

Severity of Complaints: No Symptoms (0), Mild (1) to Very Severe Complaints (5)						
Gastrointestinal						
	0	1	2	3	4	5
Abdominal pain	o	o	o	o	o	o
Intestinal colics	o	o	o	o	o	o
Bloating	o	o	o	o	o	o
Diarrhea	o	o	o	o	o	o
Constipation	o	o	o	o	o	o
Nausea	o	o	o	o	o	o
Belching	o	o	o	o	o	o
Vomiting	o	o	o	o	o	o
Postprandial fullness	o	o	o	o	o	o
Menstrual cramps	o	o	o	o	o	o
Skin						
	0	1	2	3	4	5
Pruritus	o	o	o	o	o	o
Eczema	o	o	o	o	o	o
Reddened skin	o	o	o	o	o	o
Swollen, reddened eye lids	o	o	o	o	o	o
Cardiovascular						
	0	1	2	3	4	5
Headache	o	o	o	o	o	o
Dizziness	o	o	o	o	o	o
Hypotonia	o	o	o	o	o	o
Palpitations	o	o	o	o	o	o
Collapse	o	o	o	o	o	o
Respiration						
	0	1	2	3	4	5
Rhinorrhea	o	o	o	o	o	o
Nose congestion	o	o	o	o	o	o
Sneezing	o	o	o	o	o	o
Asthma	o	o	o	o	o	o
Additional Complaints (Please List Symptoms that Have Not Yet Been Listed)						
	0	1	2	3	4	5
	o	o	o	o	o	o
	o	o	o	o	o	o
	o	o	o	o	o	o

Figure 8. A standardized questionnaire for the assessment of patients with suspected histamine intolerance (HIT). (Wolfgang J, et al., 2021)

In theory, if the cause of this inhibition or low activity of the enzyme is due to the interaction of a drug that prevents it from functioning correctly, it is conceivable that if treatment with the drug is discontinued, once the drug has been completely eliminated from the body, the enzyme will function correctly again (without affecting its active site). (23) However, if the treatment has been long-term and the effects continue to persist, there is the option of taking oral enzyme supplementation which would provide the body with undamaged DAO enzyme with the ability to metabolise histamine, which in this case is the cause of the damage. Moreover, this supplementation could be ingested without interrupting the medication treatment which may be associated with a low histamine diet.

As a proof, a trial of supplementation with DAO capsules, specifically DAOSIN, provided by Sciotec Diagnostic Technologies, Tulln in Austria (DAOSIN) was conducted in which for 8 weeks patients were not required to change either their diet or their medication. The study was divided

in two, during the first 4 weeks patients took DAOSIN capsules (4.2 mg of protein extracted from pig kidney with 0.3 mg of DAO) before meals, up to three times a day. On the other hand, for the next 4 weeks, patients were not to take this supplementation. A record was kept of both symptoms and compliance with the study instructions, i.e. whether or not the capsules were taken, and serum DAO and plasma histamine levels were also analysed at each visit, at 2-week intervals. (24)

In order to assess the symptoms of the patients, before and during the experimental period, a standardised questionnaire based on known symptoms and the four histamine receptors was administered at each visit. Four categories were named in the questionnaire, 22 symptoms in total. The categories were: GI, cardiovascular, respiratory and skin complaints. In addition, a scoring method was used for each symptom with a range from 0 (no symptom) to 5 (very intense symptom). In the meanwhile, in order to determine the level of DAO in serum, it was carried out a radio extraction assay DAO Rea 100 (Sciotec Diagnostic Technologies, Tulln, Austria). The level of histamine in the plasma was determined with an enzyme linked immunoassay, Histamin ELISA BA 10-1000 (Diagnostika Nord GmbH & Co. KG, Nordhorn, Germany). (24)

Symptoms	4 weeks of DAO supplementation		4 weeks without DAO		
	Visit 1 Mean (± SD)	Visit 2 Mean (± SD)	Visit 3 Mean (± SD)	Visit 4 Mean (± SD)	Visit 5 Mean (± SD)
All symptoms	19.6 (± 9.9)	10.6 (± 7.7)	6.6 (± 6.4) $p < 0.0001$	10 (± 8.3)	12.5 (± 9) $p = 0.002$
GI	11.2 (± 7.5)	6.1 (± 5.2)	3.8 (± 4.4) $p < 0.0001$	6.5 (± 5.9)	7.1 (± 5.3) $p = 0.0058$
Diagnostic GI	8.4 (± 5.6)	4.8 (± 4.1)	3.1 (± 3.4) $p < 0.0001$	5 (± 5)	5.4 (± 4.3) $p = 0.0312$
Cardiovascular	3.6 (± 2.8)	1.9 (± 2.1)	1 (± 1.2) $p < 0.0001$	1.3 (± 1.7)	1.8 (± 2.2) $p = 0.0783$
Respiratory	2.4 (± 2.7)	1.6 (± 2)	1.2 (± 1.6) $p < 0.0001$	1.2 (± 1.7)	2.4 (± 2.6) $p = 0.0029$
Skin	2.4 (± 3.1)	1 (± 1.5)	0.5 (± 1) $p < 0.0001$	0.9 (± 1.6)	1.2 (± 2.2) $p = 0.1289$

The significance of the change in symptoms over time was calculated using the Friedman test

DAO diamine oxidase, *GI* Gastrointestinal

Figure 9. Calculations of the mean symptoms sum-score (SD) of all symptoms during the 8-week study period. Ten gastrointestinal symptoms, including the five most diagnostic GI symptoms, four cardiovascular symptoms, four respiratory symptoms, and four skin ailments. (Schnedl W, et al., 2019)

Thus, we can observe in Figure 9 how taking DAO supplementation mitigates the symptoms from the first to the second visit, while as time passes and the patient stops taking this supplementation, the symptoms intensify again. Even so, at visit 5, the symptoms are not as intense as they were at visit 1. Consequently, the positive impact of this oral dose should be highlighted. (24)

3.3. GENETICAL DETERMINANTS

The major cause of DAO enzyme dysfunction, also known as DAO deficiency, which results in the accumulation of exogenous histamine in tissues and organs, is hereditary. (14)

Previously it has been commented that, in the human genome, DAO enzyme genetic sequence is located in a fragment on chromosome 7 (7q34-q36). This fraction of chromosome is formed of 5 exons and 4 introns, with different sequences between individual because of their genetic polymorphism. In accordance with National Center for Biotechnology Information (NCBI), it exists about 85 different variants of a single nucleotide polymorphisms (SNPs) AOC1 which is the human gene of the DAO enzyme. Seventeen SNPs are located in the exons and 3 of these polymorphisms (rs1015611, rs1049742 and rs1049793) cause a decreasing in DAO activity in the metabolism of histamine. (14)

3.4. PATHOLOGICAL DETERMINANTS

People suffering from inflammatory bowel diseases tend to suffer from DAO enzyme deficiency. It has mostly been observed in patients with colon cancer but also in the postoperative bowel. This is because, as DAO is mainly located in the small intestine, if part of the mucosa is reduced, the production area of the enzyme is consequently decreased. (12)

3.5. PHARMACOLOGICAL DETERMINANTS

In addition to the above conditions, more than 90 drugs have been reported to be implicated in DAO enzyme deficiency or low activity as they are able to block or even inhibit the enzyme. These include analgesics, antidepressants, anti-rheumatics, anti-arrhythmics, antihistamines and mucolytics, among others. The use of any of these drugs therefore increases the risk of symptoms or pathologies resulting from histamine accumulation. (12)

It is important to consider the pharmacological determinants since, as these drugs are usually prescribed to alleviate the effects of other diseases, the effect they can have is often ignored to a certain extent. (12)

3.5.1. DAO-inhibiting drugs

Several studies have shown which drugs are involved in interfering with histamine metabolism, both in vitro and in vivo. In fact, as made clear by Duch DS, et al. (1980), in a study in rats, some of these drugs are even capable of inhibiting both HNMT and DAO enzymes. (25)

In that trial in which drugs were examined in vitro for their effects on HNMT and DAO, and in vivo for their effects on histamine levels, it was found that, among the drugs studied, three were very potent inhibitors of HNMT in vitro and two others were identified as potent inhibitors of the DAO enzyme, one of them being one of the three HNMT blockers. (25)

The drugs, which in this case were classified as DAO inhibitors, were a trimethoxybenzylaminoquinazoline (TMQ, JB-11) - also an inhibitor of HNMT - and methasquin with K_i values of 4.1 and 3.7 M, respectively. (25)

On the other hand, the study by Sattler J, et al. (1985), also revealed the existence of drugs capable of modulating DAO activity. Unlike the previous one, in this case 164 substances of three hundred and forty-one drugs, commonly used in intensive care units (ICU), were examined using both canine and human DAO in an in vitro screening test. The results indicated that 61 agents inhibited DAO activity to various degrees. Of these, 13 were capable to inhibit only the human DAO, whereas 4 inhibited the canine enzyme and 44 were shown to inhibit the enzyme from both species. Representatives of all major treatment classes were among the inhibiting agents. (26)

It should be noted that, of the compounds tested, none were shown to enhance enzyme activity, but rather the opposite or no effect. In fact, it was observed that different drugs belonging to the same group (e.g. neuromuscular blocking drugs) differed in their effect with respect to DAO inhibition, some being able to inhibit, as mentioned above, while others had no effect. Then, the existence of histaminase inhibitory drugs, specifically in human DAO from healthy intestinal tissue, is reinforced. (26)

The figure below (Figure 10) shows a compilation of many of these drugs that are capable of inhibiting, to a greater or lesser extent, the human DAO enzyme in the gut.

International non-proprietary name of drugs	% Inhibition of DAO activity in the presence of the drug (*M*)		
	1×10^{-5}	1×10^{-4}	1×10^{-3}
Dihydralazine	96	100	100
D-Cycloserine	63	99	100
Pentamidine	79	95	100
d-Tubocurarine	51	82	95
Chloroquine	45	69	90
Pancuronium	29	63	88
Alcuronium	9	36	N.D.
Clavulanic acid	3	23	80
Carbocromene	2	27	65
Ascorbic acid	0	14	56
Pirenzepine	1	20	55
Fenpiverinium	0	23	54
Tetroxoprim	0	10	54
Colistin mesilate	1	8	54
Amiphenazole	0	8	52
Thiamine	0	25	44
Acemetacine	1	15	42
Aminocycline	4	16	41
Aminophylline	2	8	40
Orciprenaline	7	12	39
Metoclopramide	0	9	36
Framycetin	0	5	34
Dopamine	0	3	34
Cefuroxime	7	11	33
Dipyrone	1	3	30
Pramiverine	0	5	30
Cefotiam	1	5	29
Chloryphenoxamine	0	0	28
Ciclacillin	0	0	27
Proxyphylline	6	8	26
Clomipramine	0	0	23
Promethazine	5	0	23
Iobenzamic acid	0	1	22
Salazosulfapyridine	3	2	21
Chloropyramine	2	0	20
Acebutolol	1	2	18

Figure 10. Percentage of human DAO inhibition by drugs with inhibitory effects on human intestinal DAO. (Sattler J. et al., 1985)

In addition, this inhibitory power of certain drugs has also been demonstrated in a study focusing specifically on the effect of the drug metronidazole, an antibacterial and antiprotozoal drug used as an effective therapy for anaerobic infections and a variety of protozoan and parasitic diseases. Metronidazole has been used for the treatment of infections for a long time and is still used successfully for the treatment of trichomoniasis, amebiasis and giardiasis, including several anaerobic bacterial infections. (27)

Befani O, et al. (1995), described how metronidazole inhibits DAO activity from various assays with enzyme purified from pig kidney or homogenates of rabbit, rat and human intestine where metronidazole shows a non-competitive inhibition on human, rat and rabbit DAO, with a variability of K_i values between 2.5 x10-4 M and 10-4 M. (28)

Katane M, Matsuda S, et al. (2013), also determined the inhibitory power of the antiviral drug acyclovir (ACV) in this case. This study adds to previous studies by emphasising that acyclovir is a slow-binding inhibitor of DAO. (29) ACV is a nucleoside antiviral drug that has in vitro antiviral activity against DNA viruses belonging to the herpes family, varicella-zoster, and Epstein-Barr. (30) The present study had been performed in silico with pig DAO, human recombinant DAO and

human DDO (D-aspartate oxidase) and states the following: "Human DAO shares high amino acid sequence identity with pig DAO (84.4%), whereas human DDO shows only moderate sequence identity with pig DAO (39.2%)". It was concluded that ACV acts on DAO as a slow-binding reversible inhibitor, and the time required to reach equilibrium between DAO, ACV and the DAO/ACV complex is highly temperature-dependent. In any case, the inhibitory effect of ACV in a DAO active site-directed manner was demonstrated. (29)

In another in silico screening of recombinant human DAO, Katane M, Osaka N, et al. (2013), 15 drugs had been chosen in which they investigated whether they inhibited only the DAO enzyme or, conversely, could act by inhibiting other enzymes. Indeed, three competitive compounds that exhibited inhibition against DAO but not against other enzymes were further studied. Crotonate and 4H-furo[3,2-b]pyrrole-5-carboxylic acid are also listed as competitive inhibitors of mammalian DAO used as negative controls with benzoate too (23, 24) renaming what it already affirmed Klein, J. R., 1960, that benzoate inhibits the oxidase by competing with substrate. (33)

In addition to this drugs, the chlortetracycline (CTC), an antibiotic of the tetracycline family (34), has an inhibitory effect against DAO too. (27, 28) Also it is very important to name aminoguanidine (AG), which has been shown to have anti-inflammatory and radical scavenging effects (37) and to be an efficient agent for reducing the incidence of diabetic nephropathy-related changes. (38) In the past, Rokkas T, et al. (1990), referred to aminoguanidine treatment as complete inhibitor of DAO in the ileal mucosa in rats or at least its reduction to unmeasurable levels. At that time, although the information was limited, it was already suggested that the profile of DAO activity in the human intestine was similar to that in the rat, thus demonstrating, once again, the inhibitory effect of drugs on DAO. (39)

In 2014, the impact of active ingredients in some drugs on the activity of human DAO was quantified in a report. Leitner R. et al. (2014), made an in vitro determination and quantification by activity test: interaction with purified human DAO. They concluded that, according to the results displayed in Figure 11, chloroquine (CQ) and clavulanic acid (CA) (> 90% DAO inhibition) have a high inhibitory effect while isoniazid and verapamil (about 50%) have a medium one. Cimetidine and metamizol (35%), acetyl cysteine and amitriptyline (>20%) were situated as moderate inhibitors and diclofenac, metoclopramide, suxamethonium and thiamine (<20%) were classified as the lower ones, emphasizing that cyclophosphamide and ibuprofen did not show any effect.

	Active ingredient	Inhibition	effect
strong DAO inhibitors	Chloroquine	99%	antimalarial
	Clavulanic acid	92%	antibiotic
modest DAO inhibitors	Verapamil	50%	calcium channel blocker
	Isoniazide	47%	antibiotic
	Cimetidine	37%	antihistamine
	Metamizol	35%	analgesic/antipyretic
	Amitriptyline	33%	tricyclic antidepressant
	Acetyl cysteine	29%	mucolytic agent
Weak DAO inhibitors	Thiamin	8%	vitamin
	Metoclopramide	7%	antiemetic
no DAO inhibition	Ibuprofen	4%	NSAID
	Suxamethonium chloride	4%	depolarizing neuromuscular blocker.
	Diclofenac	2%	NSAID
	Cyclo-phosphamide	1%	Chemotherapeutic

Figure 11. Active ingredients from some drugs and its percentage of inhibitory effect against human DAO. (Leitner R. et al., 2014)

As a result, most of the compounds observed can be classified as DAO inhibitors, as even approximately 30% levels of inhibition can be significant. (40)

3.5.1.1. Why and how drugs inhibit DAO - Types of DAO inhibition

As commented in the previous section, there is confirmation of the existence of drugs that affect the activity of the DAO enzyme. In this section, the molecular mechanisms that could explain such inhibition will be described.

It could be hypothesised that there is an interference in the DAO signalling pathway. Nevertheless, all the evidence suggests that the active ingredients of the drugs actually act directly on the enzyme. Although this is usually the case, leading to a common result, not all drugs inhibit the enzyme in the same way.

According to the different types of enzyme inhibition, particularly in the diamino oxidase, drugs can act through some of this mechanisms, being all reversible inhibitors. Within this group of reversible inhibition, we can differentiate between diverse bindings. As shown in Figure 12, there are drugs that act on DAO in a competitive manner while others may do so in a non-competitive form.

	Type*
DAO	
Amodiaquine	NC
Isometamidium	NC
Antrycide	NC
Imidocarb	NC
Amicarbalide	NC
MGBG	NC
Aminoguanidine	C

* C, competitive inhibition; NC, non-competitive

Figure 12. Type of DAO inhibition by antimalarial and antitrypanosomal drugs. (Adapted from Duch DS, et al., 1984)

Many nitrogen-containing bases substances, particularly bases like amidines and guanidines, carbonyl reagents, substituted hydrazines, and chelating agents, have been shown to inhibit DAO. Many of the known DAO inhibitors are monoamine oxidase inhibitors, which are a type of drug. When such diamines are used as substrates, DAO is also subject to substrate inhibition. (42) Therefore, the presence of nitrogenous bases in the structure of drugs may be one of the causes of DAO inhibition.

In relation, the similarity between the structure of the drugs and the structure of the biogenic amine substrates of DAO may also be part of the cause of this inhibition as the drug would hold the key to bind to the site where the BAs fits.

Although the focus of this review is histamine, it should be noted that the enzyme DAO it is also involved in the putrescine catabolism and that results with putrescine in vivo seem valid for histamine too, at least for rats. (43)

In consequence, it is thought-inspiring that CQ and quinacrine, two DAO-inhibitory antimalarial drugs, contain the 'putrescine skeleton', that is to say, a 4-carbon chain between two amino groups. Another same-type drug, the amodiaquine, contains this unit too but in this case, three of the carbons in the chain are part of the phenolic group. This 4-carbon structure may play a role in drug binding to the binding site of putescine in DAO -also applicable to histamine which does not have the same structure as putrescine but similar -. (43) Both structures, putescine and histamine, are evidenced in Figure 13 and Figure 14, respectively.

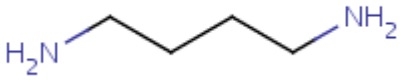

Figure 13. Structure of putrescine. (EMBL-EBI)

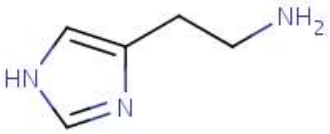

Figure 14. Structure of histamine. (EMBL-EBI)

Another interesting case is the binding of ACV to DAO which appears to be biphasic due to the effect of the temperature. It is plausible that depending if the temperature is low or high, the molecular mechanisms that make possible the union between ACV and DAO have some differences, varying in the binding affinity of ACV. Figure 15 shows the comparison between both temperatures 0 °C and 5 °C, as the temperature decreases, the affinity of ACV for binding DAO active site increases. On the other hand, comparing 37 °C with 20 °C, by contrast, as the temperature increases, the binding affinity increases too. In conclusion, temperature tends to have a biphasic effect on ACV-DAO binding. (29)

	relative activities (%)[a]		
1 h preincubation	pig DAO	human DAO	human DDO
none	84.3 ± 2.3	99.5 ± 0.3	101.4 ± 2.4
0 °C	0 ± 0	0 ± 0	72.8 ± 0.9
5 °C	70.7 ± 1.3	99.7 ± 4.2	97.6 ± 1.1
10 °C	75.0 ± 0.5	95.6 ± 2.6	96.8 ± 2.1
20 °C	75.1 ± 2.6	94.2 ± 1.3	98.7 ± 3.0
37 °C	28.6 ± 4.1	46.6 ± 7.9	93.1 ± 3.1

[a]Enzyme activity is presented as a percentage relative to the activity obtained in the absence of ACV. Each value shown is the mean ± standard deviation (n = 3).

Figure 15. Preincubation with ACV (1000 µM) on enzymatic activities (pig DAO, human DAO and human DDO). Remarked the biphasic temperature-depending binding of ACV to human DAO. (Katane M, Matsuda S, et al., 2013)

A further one mechanism of DAO inhibition by drugs is the CTC – DAO binding. Kinetic studies exposed that CTC inhibits DAO with a single mechanism by competing with the oxidase protein for the coenzyme flavin adenine dinucleotide (FAD), resulting in a complex formation of CTC with FAD which can be the cause of the inhibitory effect. (36)

Certainly, CTC is able to blend with free FAD but it can not bind with it if FAD is bounded. CTC needs an extended time to reach an efficient and constant inhibition in case of FAD has been bound before with the oxidase protein so the drug do not inhibit the initial activity of DAO. (36)

According to these findings, it seems that the binding site of FAD takes part with the complex formation between it and the oxidase protein that might be in charge of its complex formation with CTC. The direct action of CTC on FAD in vivo will result in the inhibition of flavin dependent enzymes. (36) Moreover, CTC is not the only drug in which have been seen this behaviour. In fact, decreasing concentrations of free FAD significantly lowered the inhibitory activity of ACV against DAO. As a result, these findings suggest that the FAD bound to DAO plays also a role in ACV interaction. (29)

Comment that, when compared to porcine DAO, human DAO binds FAD weakly and has a significantly slower rate of flavin reduction. The active site of human DAO, on the other hand,

was thought to be nearly identical to that of porcine DAO. This indicates that the low affinity for FAD is due to conformational flexibility that depends on the context of the hydrophobic section as well as the delayed rate of flavin reduction, emphasizing the defining characteristics of human enzyme. (46)

DAO inhibition form and order of magnitude by metronidazole are similar to those observed for this enzyme in the presence of imidazole. The hydrophobic interaction between the aromatic ring of metronidazole and a hydrophobic site of DAO is most likely to blame for the non-competitive nature inhibition concurring with the achieved results in relation to various hydrazide derivatives on Copper-dependent Amine Oxidases. (28)

Benzoate and 4H-furo [3, 2-b] pyrrole5-carboxylic acid have shown a competitive type of inhibition. The three dimensional (3D) X-ray crystallographic structure of human DAO has given the possibility to complex it with some drugs to observe how the inhibitory mechanism is and which residues are involved. (31)

In relation to the structure of human DAO complexed with benzoate, it can be appreciated that the carboxyl group of benzoate connect with DAO in two different locations. One of this is the side chain guanidino group of Arg-283 and the second one is with the side chain hydroxyl group of Tyr-228. Katane M, Osaka N, et al. (2013), hypothesized that a loop formed by residues 216−228 play an important role acting as an “active-site lid”. It is proposed that this “active-site lid”, when the movement of substrate and/or product into and out of the active site occurs, acts like a gate opening and closing. The residue Tyr-224 located into the lid is catalytically important for the full enzymatic activity of DAO when the closed conformation occurs because of the hydrophobic environment of the active site. (31)

Referring to the presence of FAD, the apoprotein (DAO) and the benzoate combine with two different binding sites of this coenzyme. Specifically, both fits with the isoalloxazine nucleus of FAD. What is more, the reality that benzoate is a substrate (D-amino-acid) competitor, denote that there is also a union between benzoate and the apoprotein. Thus, it might be contemplated that the three of them, FAD + apoprotein + benzoate, conjoin to form a close-fitting complex. (23)

It should be mentioned that benzoate acts as a brace to form this complex and that it can easily exchange by the D-amino-acid substrate, without causing any denaturation effect on the active site of the apoprotein. (23)

However, while there is no presence of benzoate, the binding of the coenzyme and substrate to the apoenzyme is considered to cause the mutual interaction between the apoenzyme, the FAD and the substrate so the substrate is activated by the enzyme turning FAD into a semiquinoid form, denoted by E'S. These interactions, particularly the change in protein conformation, are likely to be important in enzymic catalysis. (47)

$$E + S \rightleftharpoons ES \rightleftharpoons E'S' \rightleftharpoons E + P$$

$$E + B \rightleftharpoons EB$$

Figure 16. Description of conformational model of the ES (enzyme + substrate) or EB (enzyme + benzoate) complex. (Yagi K, Ozawa T, Ooi T, 1963)

On the other side, in relation to the structure of human DAO complexed with 4H-furo [3, 2-b] pyrrole-5-carboxylic acid, the carboxyl group of this drug fits with the side chain guanidino group of Arg-283 and with the side-chain hydroxyl group of Tyr-228. In addition, its heterocyclic pyrrole NH group interface with DAO in the backbone carbonyl of Gly-313. (31)

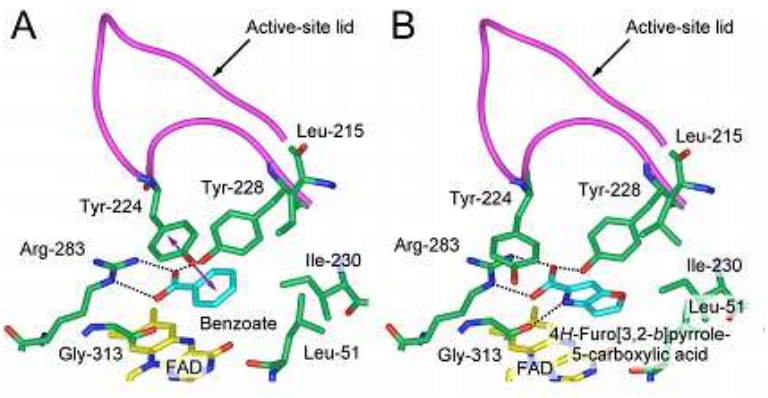

Figure 17. Experimentally determined structures of human DAO complexed with benzoate (PDB ID: 2DU8) (A) and 4H-furo [3, 2-b] pyrrole-5-carboxylic acid (PDB ID: 3CUK) (B). The carbon atoms in FAD are coloured yellow; bound ligands are cyan and side chains of amino acid residues are coloured green. Other atoms: nitrogen is blue; oxygen is red and sulfur is brown. The "active site lid", a loop formed by residues 216–228 is coloured magenta. Possible hydrogen bonds or Van der Waals interactions are represented by dashed black lines while possible π-π interactions are shown by purple arrows.. (Adapted from Katane M, Osaka N, et al. 2013)

Structural model of human DAO complexed with ACV were developed based on these experimentally defined structures. This model prophesizes that ACV binds to the active site of human DAO by diverse interactions. The hydroxyl and lactam NH groups of ACV interface with the sidechain hydroxyl group of Tyr-224 and the O4 atom of FAD, respectively. Accordingly, ACV interfere with the accurate orientation of the substrate in the active site, ending in an inhibition of the enzymatic activity. (29)

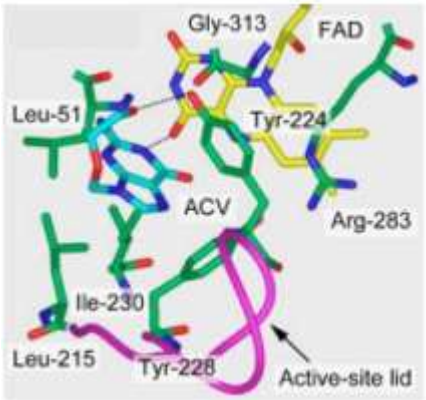

Figure 18. Human DAO complexed with ACV: a proposed structural model using Glide software. The carbon atoms in FAD are coloured yellow; bound ligands are cyan and side chains of amino acid residues are coloured green. Other atoms: nitrogen is blue and oxygen is red. The "active site lid", a loop formed by residues 216–228 is colored magenta, while possible hydrogen bonds are represented by dashed black lines. (Adapted from Katane M, Matsuda S, et al. 2013)

Therefore, the drugs-DAO bindings are not established between the same residues, as they are different structures. However, although different types of binding take place, all fit in at the active site of the enzyme.

3.5.1.2. Multifunction of DAO-inhibiting drugs

The existence of drugs with an inhibitory effect on DAO enzyme activity has been demonstrated. However, drugs have also been found to activate the secretion of endogenous histamine, i.e. histamine secreted by the own metabolism of the body.

Interestingly, some of these inhibitory drugs also have a stimulatory effect on endogenous histamine. This means that treatment with one of these drugs would not only cause a decrease in the degradation of exogenous histamine by histaminase but also promote an increase in the secretion of histamine in the body, in this case endogenous histamine. (14) As a result, since DAO is responsible for degrading exogenous and non-endogenous histamine, there is an increase in the concentration of exogenous histamine in the bloodstream of the patient, to which can be added the increase in endogenous histamine, unrelated to the activity of the DAO enzyme, but which aggravates the effects by a general accumulation of histamine.

Of these multifunctional drugs in terms of histamine, the first is acetylsalicylic acid as the active ingredient, which is an analgesic. Secondly, in this case the pharmacological indication is expectorant, and the active ingredient is ambroxal (Mucosan). Last but not least, acetyl cysteine (Fluimicil and Frenacil), whose pharmacological indication is as a mucolytic. (14)

Therefore, taking these drugs has a double aggravating effect on the patient being medicated, reaching even higher levels than those that can be caused only by enzyme inhibition.

3.5.1.3. DAO-inhibiting drugs: intentionally vs. unexpectedly

3.5.1.3.1. Intentionally antagonist effect

DAO is also involved in the oxidative metabolism of D-amino acids, such as D-serine, which is a complete agonist at the allosteric glycine binding site of the NMDA receptor. D-serine has been shown to help with negative and cognitive symptoms of schizophrenia, which are not well treated by traditional D2 antagonist treatments. (48)

In vitro, the antipsychotics DAO antagonists 3-methylpyrazole-5-carboxylic acid and 4H-thieno [3, 2-b] pyrrole-5-carboxylic acid, as well as the second-generation antipsychotics, blonanserin and risperidone, were found to have reasonably powerful human DAO-inhibitory effects. The DAO-inhibitory effects of this second-generation antipsychotics should then be considered in the light of their in vivo pharmacotherapeutic effectiveness so prescribed antipsychotic drugs can inhibit human DAO activity in vivo. (49)

This is therefore a case of intentional DAO inhibition, as the drug is intended to inhibit the enzyme as a treatment for schizophrenia. In other words, the enzyme should not be active, otherwise it degrades D-serine, which is an amino acid that reduces the symptoms of schizophrenia. On the other hand, however, inhibition of DAO can lead to other problems such as an increase in histamine and thus histaminosis.

3.5.1.3.2. Unexpectedly antagonist effect

This review focuses on these effects, as they occur by chance, as DAO inhibition is not the desired effect of the medicine. Consequently, they can lead to the development of problems in the patient or even aggravate existing problems that are being treated with this drug.

Returning to Figure 11, where it could be observed two main inhibitors of DAO: CQ (99%) and CA (92%), the first of them is an antimalarial drug whereas the second is an antibiotic. (50)

The antimalarial one is a 4-aminoquinoline drug that, as its name suggests, is used to treat or prevent malaria. For several decades, it was the most effective and least costly antimalarial in countries where malaria was endemic, and it is still recommended for treating P. vivax infections. (51) Nevertheless, it is also used in the treatment of some autoimmune diseases, such as rheumatoid arthritis or systemic lupus erythematosus. (52) By contrast, CA is a major beta-lactam antibiotic developed naturally by the bacteria Streptomyces clavuligerus and is active against a wide range of Gram-positive and Gram-negative bacteria. (50)

Contrary to the previous section, in this case we are referring to medicines in which the main activity of the treatment is not the inhibition of the DAO enzyme. In spite of, due to the interactions that might happen and possibly some of the cases mentioned as types of inhibition, the drug does affect the enzyme, producing a shortage in its activity in the body of the patient. Thus, contrary to the correct function of the enzyme DAO shown in Figure 19, there would be a high reduction of histamine degradation in the intestine resulting in a build-up of it. As established in Figure 20, the accumulated histamine could then cross the intestinal mucosa and reach the bloodstream, which distributes it throughout the organism. This is where the histamine receptors have an important role: H1, H2, H3, and H4, which are located at different sites. The high concentration of histamine in the bloodstream trigger the binding between histamine and its receptors in different areas, giving rise to various symptoms associated with DAO deficiency (Figure 21) such as gastrointestinal, skin, neurological and muscular symptoms, colon or irritable bowel syndrome, dermatitis, dry skin, atopic skin, contractures, muscle pain, migraine and headaches. (19)

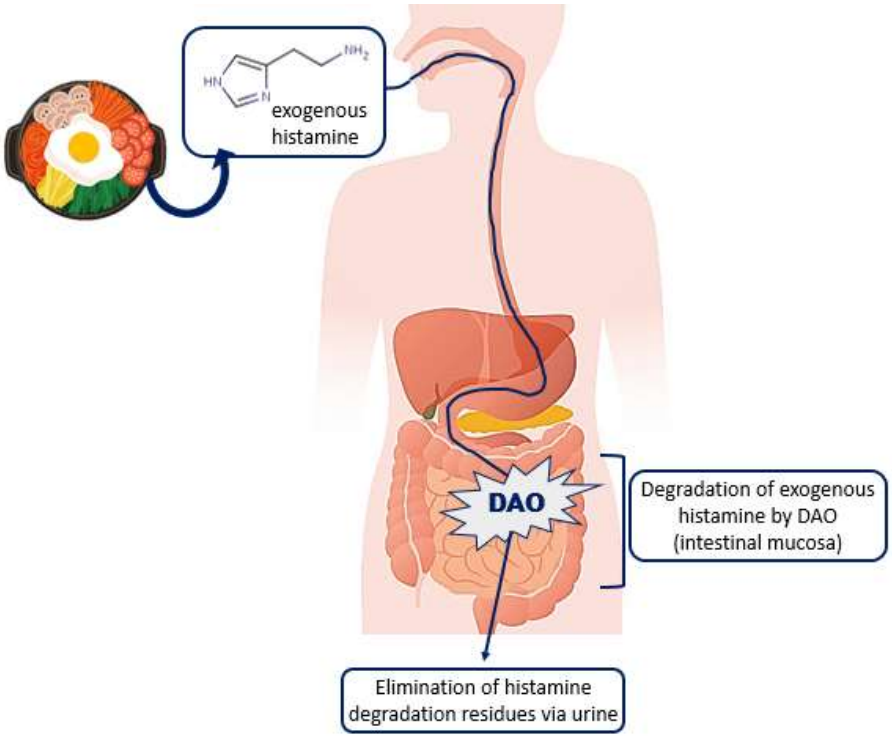

Figure 19. Correct function of the enzyme DAO. Degradation of exogenous histamine by DAO and following secretion by urine. (Own elaboration)

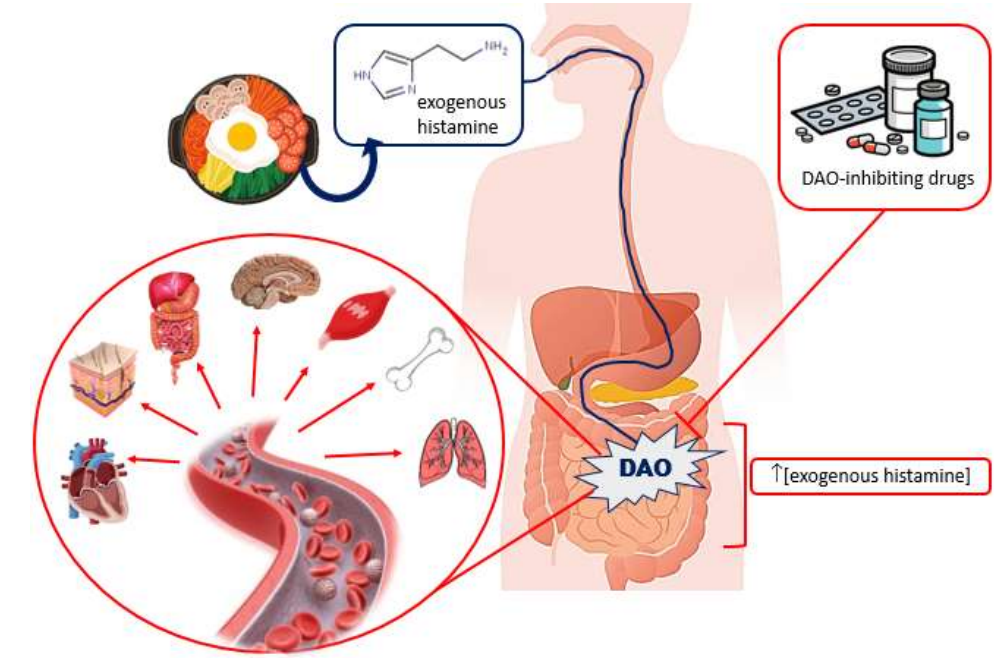

Figure 20. Interrupted DAO function by drugs. Accumulation of exogenous histamine and its crossing across intestinal mucosa to the bloodstream, reaching different organism systems. (Own elaboration)

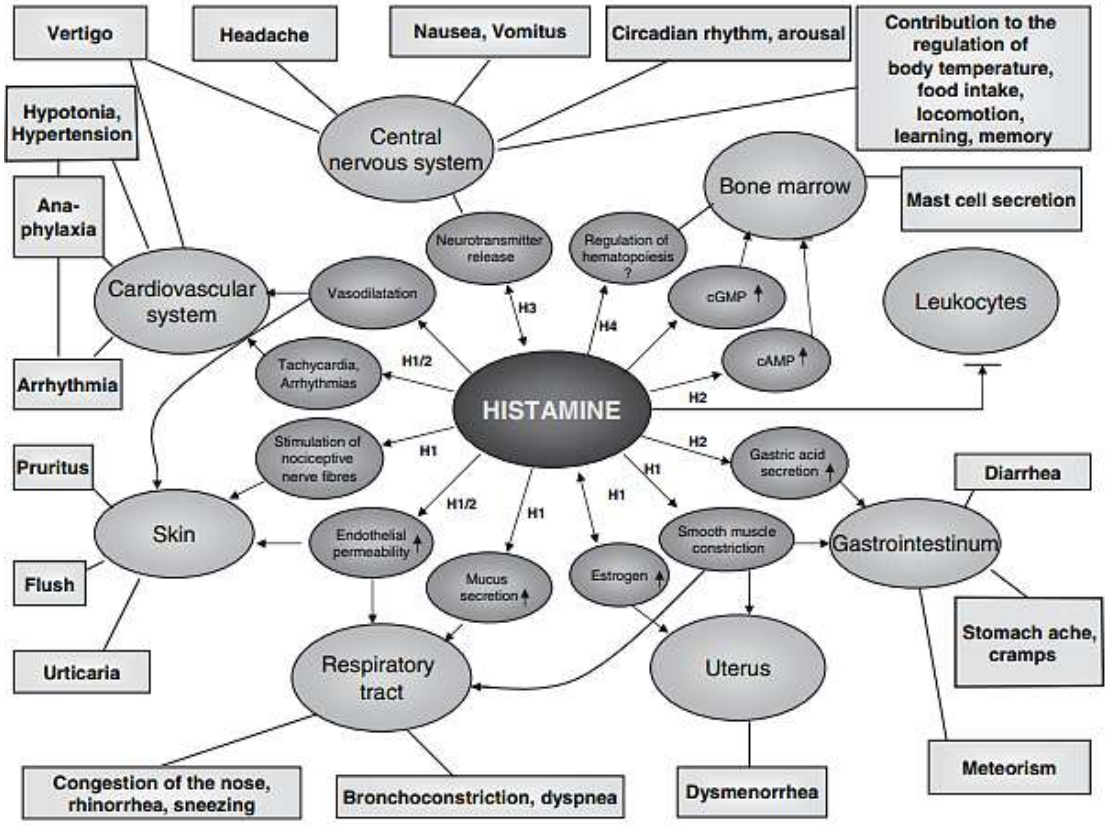

Figure 21. Condensed histamine-related symptoms. (Maintz L, Novak N, 2007)

4. DISCUSSION AND CONCLUSION

The GI tract is a complex system involving a variety of organs and their respective functions. The digestive enzymes have a particular importance, which can be affected by various environmental conditions such as diet and drugs. Although we know that these drugs are specifically designed to act on a target molecule, once the drug enters the body, it may secondarily interfere with other, usually unwanted, signalling pathways.

The adverse effects of drugs are studied and known in order to protect the population from their use. However, it seems that patients who are undergoing drug treatment and suffer from gastrointestinal problems are not tested sufficiently or perhaps adequately and therefore do not have a clear diagnosis of their symptomatology. As a consequence, there is a certain neglect of this part of the population.

Nowadays, many people suffer from gastrointestinal problems, with generally common symptoms among them what has motivated me to carry out this research. Food intolerance or non-immunological reaction tends to go unnoticed among the population, without being given the attention it deserves. Within this group, intolerance to BAs may appear to be controlled by not exceeding its intake. However, if we focus on histamine, it is not the only drawback. DAO, which is responsible for degrading exogenous histamine, plays a very important role in preventing the accumulation of this BA and therefore avoiding the possible adverse effects of its accumulation in the body. The downside comes when this enzyme, which is mainly found in the intestine, loses its enzymatic activity and therefore stops working or has a slow activity. This leads to an increase of exogenous histamine in the organism. As soon as histamine is able to cross the intestinal mucosal barrier and reaches the bloodstream, it is distributed activating various histamine receptors and causing undesired symptoms. Symptomatology is varied, however, it seems that studies have given much importance to migraine as a consequence of histaminosis, leaving aside other important ailments such as those of the GI system.

In people suffering from these complaints, in most cases, DAO deficiency is not even considered as an option, which could contribute to the detection of the pathology suffered by the patient in question, as in many cases this would probably be the cause. As a consequence, the persistence of symptoms is an aggravating factor for mortality and also has an impact on healthcare costs. It is therefore advisable to consider DAO deficiency when making a diagnosis that comply these characteristics.

The investigation performed in this review indicated that, independently of the genetic and pathological determinants that can cause DAO deficiency, taking certain common drugs such as CQ and CA can also have this effect on this enzyme. While it is true that some drugs are specifically designed to inhibit DAO - antipsychotic drugs - because the treatment of some diseases such as schizophrenia requires it, others do so as an adverse effect. As a point in favour of these drugs, it has been determined that their inhibition of DAO is reversible and, although they all have similar interactions but with different residues of the enzyme, they always act on the active site. Then, when the administration of the drug is stopped, the active site will no longer be inactive and the enzyme will continue to function correctly. The problem comes when a treatment is prescribed without considering this side effect and, in addition, once the characteristic symptoms of histaminosis appear in the patient, the drug intake is not considered as the main cause. This point could be a setback in the recovery of patients, adding to the pathology for which they are following such treatment, the effect caused in an adverse way.

For these reasons, I believe it is important to be aware of the existence of this type of drugs and to continue the line of research deeping into how they affect the human organism. Furthermore, a proposal could focus on the search for new drugs that perform the same main function as the

aforementioned drugs but reducing the adverse effect of DAO inhibition. This could be based on isolating the active ingredient and varying the general structure of the drug, thus avoiding structures similar to those of the enzyme ligand and achieving, in the best case, a drug that does not inhibit DAO by chance.

As a final conclusion, I would like to point out that the consumption of drugs with a non-primary capacity to inhibit the intestinal DAO enzyme can lead to gastrointestinal health problems that get worse the situation of the patient, as well as other equally aggravating effects in other areas of the body. An immersion in the study of the subject can not only help and improve the health of citizens who are already symptomatic, but also prevent the appearance of ailments in those who have not yet started any kind of treatment.

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