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EFFECTS OF A PLANT AMINE OXIDASE IN ALLERGIC AND HISTAMINE–MEDIATED CONDITIONS

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Abstract

This review provides an update on histamine, on diamine oxidase (**DAO**) and on their implications in allergy and various conditions or affections, such as food histaminosis and inflammatory bowel diseases (**IBD**). The review also presents, in brief, patent coverage on therapies for allergy and IBD with the focus on histamine-related treatments.

Keywords: Amine oxidase, anaphylaxis, bowel diseases, cell proliferation, diabetes, histamine, inflammation, ischemia- reperfusion, polyamines.

Introduction

Amine oxidases (AOs), ubiquitarian enzymes widely distributed among living organisms [1], catalyse the oxidation of monoamines and polyamines [2]. Histamine and other primary amines released during anaphylactic reactions, undergo oxidative deamination by AOs with release of aldehyde, ammonia (NH_3) and hydrogen peroxide (H_2O_2). AOs can be divided into two classes, depending whether if the prosthetic group is flavin adenine dinucleotide (FAD) or 2,4,5-trihydroxyphenylalanine quinone (TPQ), a cofactor derived from the post-translational oxidation of a tyrosine residue. This second group of enzymes ($CuAO_S$, EC 1.4.3.6) contains copper and TPQ. Among the Cu-TPQ class, plant CuAOs enzymes are, in general, more efficient than the animal ones, probably because they function through a radical mechanism which does not operate in animal CuAO enzymes [3].

Many physiological functions are ascribed to AOs. Although the exact molecular mechanism of the biological activity is not completely defined, a role of these enzymes in various diseases has been postulated. The patho-physiological relevance of histamine, polyamines, hydrogen peroxide and aldehydes in cell differentiation, cell death, allergic diseases and post-ischemic reperfusion damage was studied. Furthermore, a relationship between the plasmatic activity of benzylamine oxidase (Bz-AO) and diabetes has been described [2].

Role of polyamines and diamine oxidases in cell proliferation and differentiation

High polyamine levels are typical for actively proliferating cell populations, while the reaction products are involved in opposite effects, impairing cell growth and proliferation *in vitro* and *in vivo* [2]. In fact, when the polyamine level is decreased by difluoromethylornithine, an inhibitor of ornithine decarboxylase, cell proliferation stops, and starts again when the levels of polyamines increase, indicating that the high level of polyamines is a *causative* event and not a result of cell growth [4]. Cell growth and

proliferation are impaired, *in vitro* and *in vivo*, in the presence of oxidized polyamines [5], and the aldehydes produced by their oxidation are involved in the inhibition of nucleic acid and protein synthesis [6]. Pig kidney DAO, immobilized on Concanavallin A-Sepharose, injected intraperitoneally in mice with Ehrlich ascite tumor, inhibits cell growth probably through the formation of toxic aldehydes and/or H_2O_2 produced during oxidation of polyamines; a similar effect was observed in cultured Chinese hamster ovary cells treated with bovine serum amine oxidase (BSAO) and spermine [7]. Both aldehydes and H_2O_2 contributed to the inhibition of proliferation, at different levels and times. The presence of catalase abolished cytotoxicity in the first 20 min, whereas aldehyde dehydrogenase (ADH) protected over a period from 30 to 60 min. When catalase and ADH were present in the reaction medium, cytotoxicity was completely abolished for a period of 60 min, indicating that H_2O_2 is the reaction product contributing to the early stage of cytotoxicity, while aldehydes have a toxic effect with longer incubation times. High temperatures enhanced cytotoxicity induced with BSAO incubation, due to an increased reaction time. Based on this information, the involvement of biogenic amine oxidation products in the hyperthermic sensitivity of tumor cells can be postulated [8].

In summary, BSAO and, in general, AOs could be considerd key enzymes in cell growth and differentiation processes, and, therefore, in growth regulation and/or tumor development. DAO activity increases up to 1,000-fold in the serum of pregnant women indicating a possible protecting role against the release of polyamines from the feto-placental unit. During pregnancy, plasma semicarbazide-sensitive amine oxidase (SSAO) is significantly elevated, while in premature births a decrease of placental AO activity was found [9].

A correlation between the grade of tumor malignancy and AO activity was evidentiated in certain tumors, such as astrocytomas. However, in some patients with metastatic tumors, the activity of circulating DAO remained unchanged. Data are also available on LoVo wild type (LoVo WT) and drug resistant (LoVo DX) colon carcinoma cells, where polyamines were elevated. The results showed that H_2O_2, produced by spermidine oxidation induced by AO, was the most important factor responsible for cytotoxic effects on both cell lines. Drug resistant cell line was more sensitive to H_2O_2 and aldehydes, and this effect was not prevented by glutathione and other protective agents. The different enzyme patterns, involved in the cell defence against H_2O_2 and reactive oxygen species (ROS), should explain the different sensitivity to H_2O_2; moreover, in the tumor cells without catalase, the sensitivity to H_2O_2 is related to the cellular levels of glutathione peroxidase, glucose-6-phosphate dehydrogenase, gluthione peroxidase and reductase [10]. A direct relationship between DAO activity and tumor progression was shown by Kusche *et al*. [11] with azoxymethane treatment combined with the inhibition of DAO with aminoguanidine. After 52 weeks, none of the animals treated with azoxymethane was found to bear a tumor, while in the group treated with DAO inhibitor, tumors were detectable after 24 weeks. These evidences clearly indicate that AOs should be considered regulatory enzymes between biosynthetic and catabolic reactions.

Antiallergic and anti-anaphylactic effects of diamine oxidases

Histamine plays a fundamental role in anaphylaxis, and is involved in allergic and pseudoallergic reactions. Differently from other metabolic pathways, histaminase activity is not directly up-regulated by endogenously released histamine. Plasma histaminase activity increases in anaphylactic shock, but not during histamine injection [12]. In some cases, histaminase plasma levels are intrinsically low, predisposing

to anaphylactic reactions. Enhanced histamine levels in humans could be related to various endogenous and/or exogenous factors. Food-induced histaminosis has been described as the result of high histamine content or histamine releasers present in food [13, 14]. The first symptom of excessive histamine intake and/or release is an increase in gastric secretion followed by tachycardia, headache and hypotension [15, 16]. A large amount of histamine is found in fermented foods, such as cheese, red wine, tinned tuna fish, fish sauces, sauerkraut, cured pork and sausages, oriental food and french cheeses, where concentrations higher than 800 µg/g can be found. Chemical *de novo* formation of amines may also occur during normal cooking and food storage [17]. The concept of pseudoallergic reactions to food, or false food allergies, was first suggested by Dukor *et al.* (1980), where the abnormal intake of biogenic amines, like histamine and tyramine, is one of the major mechanism involved [18]. The main clinical symptoms are represented by a drop of blood pressure, angioedema, headaches, alterations of intestinal functions skin, and respiratory symtoms. High levels of histamine may also derive from the bacterial flora of the colon, especially in subjects with colonic dysmicrobism due to excessive intake of foods rich in cellulose and starch. Beside the excess of histamine, other histamine-dependent mechanisms of pseudoallergic reactions to food may include changes in the activity of the enzymes involved in the metabolism of histamine. A drop in histaminase and monoamine oxidase B (MAO B) levels was found in patients with atopic dermatitis [19]. Histaminase may also be inhibited by food toxins, sodium nitrite, antibiotics (clavulanic acid), and viral hepatitis [20]. Reduced DAO activity and histamine catabolism seem to be involved in episodes of bronchoconstriction occurring after intake of red wine or histamine-rich food, and intestinal DAO is thought to be required for clearance of biogenic amines [21]. Elevated mucosal histamine content and secretion were observed in the gut of patients with allergic enteropathy, as well as in patients with Crohn's disease and ulcerative colitis. In particular, there is evidence that mast cell-derived inflammatory mediators, including histamine, play a major pathogenic role in these diseases [22].

The above findings indicate a possible therapeutic use of plant-derived histaminase, characterised by highly specific activity and ability to degrade various biogenic amines, including histamine and polyamines, in the treatment of allergic and pseudo-allergic diseases.

Plant-derived diamine oxidase

Plant diamine oxidase (also known as histaminase, EC 1.4.3.6) is physiologically involved in oxidative deamination of various biogenic amines. Vegetal DAO is the most abundant soluble protein detected in the extracellular fluids of *Fabaceae*, in particular, in pea (*Pisum sativum*), lentil (*Lens culinaris*), and chickpea (*Cicer arietinum*) seedlings [23]. The plant histaminase differs from the mammalian and procaryotic enzymes in a number of peculiar features, mainly high turnover rate of catalysis, high binding affinity for histamine and high chemical stability. Moreover, this enzyme can be isolated to a high degree of purity with two simple and inexpensive chromatographic steps. An international Patent (n. PCT/EP01/13770) has been taken for a drug based on plant-derived histaminase for the treatment of histamine-mediated diseases, such as cardiac anaphylaxis, allergic asthma, allergic and septic shock, urticaria, rhinitis and conjunctivitis [24]. The main sources of histaminase are etiolated seedlings of leguminous plants, such as *Pisum sativum*, *Lens culinaris*, *Cicer arietinum*, and *Latirus sativus*, where this enzyme is present in high concentrations, up to 4% of total protein content.

Plant DAO/histaminase modulates allergic and anaphylactic responses

The massive release of histamine from tissue mast cells, elicited by the cross-linking of antigen with IgE bound to FCε receptors at the cell surface, is considered the paramount event in type I allergic reactions. The most commonly used experimental animal models for a type I allergic reaction encompasses cardiac anaphylaxis *ex vivo* and asthma-like reactions in sensitised guinea pigs *in vivo*. Pig kidney DAO has been shown to have antihistaminic activity *in vivo* and a protective role in guinea pig anaphylactic shock [25]. The protective effect of purified pea seedling histaminase, both free and immobilised on CNBr-Sepharose, in active cardiac anaphylaxis has been observed [25]. Briefly, guinea pigs were actively sensitised and 2-3 weeks later, the heart was removed and perfused in a Langendorff apparatus, which allows an accurate determination of heart rate, contraction strength and coronary flow. Administration of antigen to the isolated hearts induced typical histamine-related changes in cardiac function. These changes consisted in a transient increase followed by a long-lasting reduction of myocardial contractility, an increase in heart rate and occurrence of severe arrhythmias and, a marked reduction in coronary flow. When antigen-challenge was performed in the presence of free or immobilised histaminase, the positive inotropic and chronotropic responses were fully blocked, including a dramatic decrease in the occurrence of ventricular arrhythmias and of the levels of histamine in the perfusates. As expected, histaminase preparations did not reduce mast cell degranulation in response to the allergen, presenting any chromoglycate-like effect on anaphylactic degranulation of mast cells, clearly demonstrating that the cardioprotective effect of free or immobilized histaminase was chiefly dependent on the inactivation of the released histamine [25]. Moreover, antigen-challenge caused a slight decrease in cardiac cGMP and an increase in tissue Ca^{2+} levels, both effects being prevented by histaminase treatment. The mechanisms underlying these protective effects remain a matter of speculation, but we can correctly hypothesize that this effect could be mediated by an interaction with the nitric oxide (NO) synthase (NOS) pathway, since histaminase significantly increases cardiac NOS activity [25] and NO stimulates cardiac cGMP levels and decreases tissue Ca^{2+} concentrations [26].

Allergic asthma is a major respiratory disease with a markedly increasing morbility worldwide. Despite decades of research, the pathogenic mechanisms are not completely understood; nevertheless, there is general agreement that histamine is a crucial mediator of the inflammation cascade and bronchospasm, two key features of allergic asthma. In this context, an experimental study to evaluate the effects of histaminase, free or immobilised, on asthma-like reactions induced in antigen-sensitised guinea pigs by aerosolic exposure to the allergen has been published [27]. This animal model is known to reproduce respiratory abnormalities, airway hyperresponsiveness and leucocyte lung infiltration resembling to the functional and histopathological hallmarks of human allergic asthma [27, 28].

Histaminase, free or immobilised on BrCN-Sepharose, was injected intraperitonneally (i.p.). The pharmacokinetic profiles showed higher amounts of histaminase in lungs from animals treated with the immobilised enzyme than in those treated with the free enzyme, and lower blood levels. The unexpected higher amounts of immobilised histaminase rather than free histaminase in the lungs could be ascribed to the fact that homing of the enzyme conjugate in those organs could be facilitated by the presence of galactose units in the molecule of the Sepharose vehicle. Challenge of ovalbumin-sensitised guinea pigs with aerosolised antigen resulted in severe abnormalities in their respiratory pattern, consisting of reduced latency time for the appearance of cough, increased cough severity and increased occurrence of dyspnaea

and gasping, indicating the onset of respiratory failure. Pretreatment of animals with histaminase, i.p. or as aerosol solution, resulted in a marked reduction of breathing abnormalities and prevention of respiratory failure. Histaminase treatment also prevented the histopathological lung changes induced by antigen-induced bronchospasm and reduced the inflammatory process characterized by leucocyte infiltration and oxygen free radical generation. In fact, histaminase decreases myeloperoxidase activity, a marker of tissue leucocyte infiltration, and malonyldialdehyde production, a marker of peroxidation of cell membrane lipids by ROS [27]. No relevant differences in pharmacological potency were noted between free and immobilized enzyme.

Plant DAO/histaminase has a protective effect against ischemia-reperfusion injury

Besides its pivotal involvement in anaphylaxis and allergic reactions, histamine plays a role in exacerbation of inflammatory tissue damage, like many pro-inflammatory mediators, such as prostanoids and cytokines that are potent mast cell activators. In particular, histamine has been found to contribute to endothelial dysfunction, hampering of blood-tissue exchange and functional tissue impairment occurring in organs undergoing ischemia and reperfusion. On these grounds, we hypothesized that pea seedling histaminase could effectively prevent the adverse effects of cardiac and intestinal ischemia-reperfusion. In fact, the heart is particularly susceptible to tissue damage by several key agents involved in the pathophysiological mechanisms of ischemia-reperfusion, such as ROS, histamine and NO [29, 30]. During acute myocardial ischemia, the coronary sinus histamine concentration increases simultaneosly with the development of early arrhytmias [31]. Histamine released from cardiac mast cells can react with the medium, provoking coronary spasm and contributing to myocardial infarction [31, 32]. Moreover, an increase of plasma levels of AOs in congestive heart failure was found [33]. A close relationship between histamine, acting as a pro-oxidant, and ROS was observed in patients with coronary heart disease [30]. In turn, histamine release by mast cells is amplified by excessive superoxide generation and concurrent decrease in local amounts of NO, due to the fact that superoxide and NO react promptly, giving rise to harmful peroxinitrite [29]. Moreover, histamine *per se* induces a spasm of the coronary vessels, thereby causing or worsening acute coronary perfusion and myocardial ischemia. Histamine levels in the coronary sinus were increased by the occurrence and severity of ventricular arrhythmias. Pea seedling histaminase demonstrated cardioprotective effects against post-ischemic reperfusion damage, in the *in vivo* model of ischemia-reperfusion in the rat. Briefly, anaesthesisedand artificially ventilated rats were subjected to 30 min of ischemia by the temporary occlusion of the left anterior descending coronary artery, followed then by 60 min of reperfusion.DAO., given intravenously either 10 min before reperfusion or exactly at reperfusion time (dose of 80 IU/kg b.w.), did not modify the at-risk area whereas the necrotic area was significantly reduced, and no cardiac protection was afforded by the semicarbazide-inactivated histaminase [34]. The number of animals which survived the 60 min-reperfusion was higher in the histaminase-treated groups than in the untreated one, the analysis of ECG recordings showing that histaminase reduced the occurrence of ventricular arrhythmias. DAO also reduced the biochemical tissue alterations induced by ischemia-reperfusion. Particularly, lung leucocyte infiltration, myocardial calcium overload and caspase-3 activation, a trigger enzyme for the activation of apoptotic cascade, were significantly reduced [34]. On these grounds, we hypothetized that pea seedling DAO could also effectively protect against the adverse effects of ischemia reperfusion of other organs, such as gut. To further validate our hypothesis, experiments on a rat model of

splanchnic artery occlusion and reperfusion (SAO/R) were carried out.

Intestinal ischemia may result from impaired blood supply to the bowel by different causes, including cardiac insufficiency, sepsis, vaso- and cardio-depressant drugs and complications of long-lasting surgery [35]. Consequences of intestinal ischemia range from persistent bleeding and symptomatic intestinal strictures to bowel perforation and peritonitis. Surgical resection of the affected bowel segment is usually required to minimize adverse outcomes. The pathophysiology of intestinal ischemia has been widely investigated in laboratory, animals undergoing surgical occlusion of the splanchnic circulation being followed by reperfusion. This results in intestinal injury and circulatory shock, characterized by severe hypotension and hemoconcentration, associated with a high mortality rate [36]. It has been shown that the endothelial dysfunction plays a key role in intestinal ischemia, as it predisposes to vasospasm, platelet activation and increased neutrophil adherence, which exacerbates the local bowel injury as well as the general cardiocirculatory failure [37]. Endothelial-leukocyte interaction is known to involve specific surface glycoproteins known as endothelial cell adhesion molecules (ECAMs). The ECAMs include early-phase molecules, such as P-selectin, involved in leukocyte tethering and rolling, which is rapidly translocated from the Weibel-Palade bodies to the endothelial cell surface upon stimulation by histamine, hypoxia and ROS [38, 39], as well as late-phase molecules, such as E-selectin, vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1, involved in leukocyte adhesion and extravasation into the tissue, whose expression is induced by most inflammatory mediators, including histamine and cytokines [40, 41]. At reperfusion, neutrophil accumulation and activation in the ischemic bowel results in the local generation and release of free radicals, lysosomal hydrolases and chemotactic factors such as leukotrienes [42], which give a major contribution to tissue injury by causing further endothelial damage and leukocyte recruitment [43, 44], plasma membrane peroxidation and DNA damage [45]. The activation of DNA repair enzyme poly-ADP-ribose (PAR) synthetase can exacerbate intestinal mucosal injury and dysfunction [46]. Free radical-mediated bowel tissue injury is largely attributable to peroxinitrite, the reaction product of superoxide anion and NO, a potent cytotoxic and pro-inflammatory molecule [29]. Moreover, a synergism between ROS and histamine acting as pro-oxidant was also observed in ischemic diseases [31]. The release of histamine from mast cells in the extracellular compartment and in blood is increased by excess superoxide anion and concurrent decrease of NO [47] and contributes to lethal circulatory shock occurring upon SAO/R. Histamine is released by the intestinal mucosa mostly during reperfusion [48] and generates a vicious cycle that leads to further endothelial activation and leukocyte adhesion and extravasation. Moreover, along with the release of histamine, the activity of DAO is significantly reduced after 60 min ischemia [49]. In keeping with these findings, mast cell-deficient mice (W/Wv) have been shown to be refractory to SAO/R-induced mucosal dysfunction [50]. In an anesthetised rat model of 45 min splanchnic ischemia followed by 6 hours reperfusion, free histaminase (80 IU/kg b.w) given 15 min before reperfusion, significantly reduced the drop in blood pressure and high mortality rate caused by SAO/R. Histaminase also reduced histopathological changes, leukocyte infiltration and expression of endothelial cell adhesion molecules in the ileum [51]. Beside reducing local tissue inflammation through acceleration of histamine catabolism, histaminase seemed to counteract ROS-mediated tissue injury, as indicated by the significant decrease in the tissue levels of peroxidation and nitration products, such as oxidized rhodamine, malonyldialdehyde and nitrotyrosine, of DNA damage markes, such as 8-hydroxy-2'-deoxyguanosine and poly-ADP-ribosylated DNA, and by consumption of tissue antioxidant enzymes, such as superoxide dismutase. As a result, histaminase lead to

a reduction of ileal cell apoptosis, as assessed by the determination of caspase 3 activity, and of the number of TUNEL-positive cells [51, 52]. As in the findings from cardiac ischemia-reperfusion, histaminase can afford protection against SAO/R-induced splachnic injury, probably due to oxidative catabolism of pro-inflammatory histamine as well as due to its antioxidant effects [51]. This results in hindrance of free-radical-mediated tissue injury, endothelial dysfuction and leukocyte recruitment. A schematic presentation of the mechanisms involved in the pathogenesis of SAO/R and on the protective effects of histaminase is shown in Fig. (**1**). A further explanation regarding the mechanism of protection by DAO could be the effect of this class of enzymes on the modulation of K^+ channels in the cells [53]; in fact a K^+- channel opener protects cardiac mitochondria by decreasing oxidant stress at reperfusion [54].

Role of amine oxidase in diabetes

The significance of increased plasma levels of benzylamine oxidases (BzAOs) in patients with diabetes is far from being clear. In diabetes, increased plasma BzAO is correlated with glycated haemoglobin [55], and the authors proposed it as an independent marker for heart failure mortality [56]. These observations suggested a relationship between plasma BzAO enzyme activity, severity of diabetes and its cardiovascular complications. Insulin-sensitizing drugs reduced plasma BzAO, and this effect is part of the beneficial effect of these drugs. However, it is not known if plasma AO levels increase following diabetogenic drug therapies. The available information is the prominent favourable role of BzAO inhibitors in reducing the severity of diabetes-related cardiovascular complications [57].

The activity of membrane-bound AOs spreads toxic aldehydes in the microenviroment that may initiate a deleteriuos cycle involving protein and DNA cross linkage related to angiotoxicity [58], a typical consequence of hyperglycemia and diabetes complications. Aminoguanidine, an inhibitor of BzAO and of NOS, has been added to common antidiabetic treatments, as a strategy to decrease the amount of glycated haemoglobin and the extent of protein-aging associated with the disease [59, 60]. In particular, in adipocytes and vascular smooth muscle cells, AO substrates generate a favourable microenvironment to remove local insulin resistance, but remain inactive with regard to the control of plasma glycemia. Pharmacological treatment of insulin resistance represents a first line approach to reduce diabetes-related complications. In recent years, many efforts focused on developing new drugs with fewer side effects, such as vanadium compounds, which lowered glycemia and normalized plasma lipid levels in an animal model of diabetes [61]. A novel combination of AO substrate and vanadium in low concentration has been proposed to increase the formation of peroxivanadate, an insulin-mimetic compound [62].

In conclusion, the control of therapeutic potential of AOs represents a field of growing interest in pharmacological research. The therapeutic potential of plant-derived histaminase in allergic diseases and anaphylactic shock and its ability to inhibit activation of a ROS-mediated inflammatory cascade and tissue injury has been investigated [51].

Recent Patent Application

Recently, an International Patent Application [24] proposing a histaminase-based drug of vegetable origin for the treatment of histamine-mediated conditions has been described. Compared to DAOs of mammalian origin, plant DAOs display: (i) high turnover rates of catalysis and high rates of reduced enzyme re-oxidation, (ii) high binding affinity for histamine (iii) and high stability. A plant DAO is currently on sale at

MoLiRom srl, Rome, Italy. However, plant proteins, due to their phylogenetic distance from mammalian sources, generally exhibit a great difference in their primary sequence and consequently high immunogenicity. In order to overcome this problem and to increase the *in vivo* residence time, grass pea DAO has been modified using four activated PEGs with different molecular weights and shapes: 5 kDa PEG– Nle–OSu, 10 kDa PEG–pNO_2 and 20 kDa PEG2–OSu. The modification reaction was carried out using various polymer/protein molar ratios. Gel filtration analysis of the reaction mixture revealed that a conjugate devoid of unmodified protein was obtained at 350:1 polymer/protein molar ratio. Under these conditions, about half of the amino groups were modified. This degree of modification could not be increased irrespective of the PEG molecular weight or the value of PEG/protein ratio, suggesting a limited availability of the amino groups at protein surface. This hindered PEGylation may be ascribed to the presence of sugars on the protein surface and/or to the dimeric structure of DAO that prevents, for steric reasons, the access of the polymer to all of the protein amino residues. PEGylation did not decrease the DAO activity, but, on the contrary, a significant increase ($P \leq 0.05$), even if slight, was observed at high PEG conjugation values.

The immunological properties of either native or modified DAO, the ability to elicit anti–DAO IgG and IgM immunoresponse after mouse boosting with native DAO, PEG 5-DAO, PEG 10–DAO and PEG2 20-DAO were evaluated. This comparative study was carried out with the conjugates obtained by extensive modification with similar amino group derivatisation. These derivatives were expected to present the best immunological properties since the high polymer mass on the protein surface could mask efficiently the protein structure. IgG and IgM were selected as immunogenic biomarkers directly correlated to the protein immunogenicity. Native DAO is a highly immunogenic as its administration elicited high anti DAO IgM and IgG immuno-response following the second immunization. The 5 kDa and 10 kDa PEG derivatives elicited a slower immunoresponse and the maximal IgM concentration reached after the 5^{th} immunization was about 50% and 80% lower, respectively, compared to the native protein. The best result was obtained with PEG2 20-DAO, eliciting only a negligible immunoresponse. The anti-DAO IgG profiles confirm the high immunogenic properties of this protein. The native protein induced maximal anti-DAO IgG concentration after 3 immunizations. PEG 5-DAO and PEG 10-DAO induced a slower immunoresponse, and the antibody generation was about 95% lower than that obtained with the native protein. Also in this case the immunogenic character of PEG2 20-DAO was negligible.

The pharmacokinetic behaviour of native and of the most promising PEGylated DAO was evaluated by determining the time of enzymatic activity in serum after a single intravenous administration to mice. Interestingly, PEG2 20-DAO exhibits a significant increase in circulating life with respect to the native enzyme, as the half-life was about 10 min for the native enzyme and 3 hours for the conjugate. In both cases, the experimental data were found to fit a biexponential behaviour indicating that either the native or the modified enzyme undergoes a distribution into a peripheral compartment. The pharmacokinetic parameters calculated by the elaboration of the experimental data highlighted the differences between the two enzyme forms. The values of α and β phase half-lives (t1/2α and t1/2β, respectively) showed that PEGylation induces a dramatic prolongation of both distribution and elimination phases. The prolonged permanence of the PEGylated DAO in the blood stream was also demonstrated by the reduced clearance (Cl), which, in the case of the PEG2 20- DAO, was about ten times smaller compared to the native protein. We can conclude that, due to the properties of this enzyme, in particular after conjugation with high mass PEG, new treatments for histamine-related disorders can be envisaged [63, 64]. Moreover, *P. sativum*

copper amine oxidase has been used as a new biocatalytic route for the synthesis of N-amidino-2-hydroxypyrrolidine. For the fist time, N-amidino-2-hydroxypyrrolidine, the product of agmatine oxidation by *P. sativum* copper amine oxidase, has been identified and characterized from structural and biochemical viewpoints. Notably, the enzymatic oxidation of agmatine leads to the cyclic compound N-amidino-2-hydroxypyrrolidine, as the only detectable reaction product. In fact, the formation of 4-guanidinobutyraldehyde was never observed. N-amidino-2-hydroxypyrrolidine inhibits competitively NOS-I, NOS-II and trypsin. This compound binds to the Glu597 and Glu371 carboxylate, present in NOS-I and NOS-II, respectively (Glu296 in human NOS-II, which is required for substrate (*i.e.* L-arginine) recognition. Moreover, N-amidino-2-hydroxypyrrolidine binds to the trypsin specificity subsite S1 forming a salt bridge with the Asp189 carboxylate. The latter is required for position recognition of substrates and inhibitors of trypsin-like serine of the cationic amino acid residue present at the P1 proteinases. N-amidino-2-hydroxypyrrolidine and agmatine displace efficiently [^{3}H] clonidine from I1-R present in heart rat membranes. Interestingly, different physiological roles (*i.e.* neuronal neurotransmission and hypotensive protection of cardiovascular system) have been linked to agmatine, which has been reported to be the endogenous ligand for I-R1 and to represent the N-amidino-2 hydroxypyrrolidine precursor. In this respect, pleiotropic functional role(s) of N-amidino-2 hydroxypyrrolidine may be envisaged, as reported for agmatine. Agmatine oxidation by *P. sativum* copper amine oxidase may therefore represent a new biocatalytic route for the synthesis of N-amidino-2-hydroxypyrrolidine, possibly representing a lead compound for the development of NOS and trypsin-like serine protease inhibitors. Moreover, N-amidino-2-hydroxypyrrolidine may represent a new ligand for I1-R [65, 66].

Recently, a plant copper amine oxidase has been used to develop a novel and visual test for oral malodour [67]. Until now, the application of biogenic amines as bio-markers of oral malodour has been limited because of the complexity of their detection. This study explores the usability of a simple colorimetric reaction detecting amines in saliva as an adjunct test for the diagnosis of oral malodour. The colour reaction caused by a newly discovered enzyme capable of detecting amines in saliva was characterized *in vitro*. Two colour scales were developed by transforming the colours of selected dilutions of a mixture of cadaverine and putrescine into a 5- and a 10-point pink-colour scale. Afterwards, this new enzymatic test was used to assess the amount of amines in saliva samples of 50 volunteers with different degrees of oral malodour. The enzymatic reaction was shown to be linear towards the concentration of amines and stable over a time of 4 h. Colour scores correlated well with organoleptic scores and also with methyl mercaptan and total sulfur compounds (total VSC).

The presented new test offers two main advantages: the possibility of confirming the presence or absence of bad breath without the necessity of having an extra device that requires maintenance and calibration, and an alternative to overcome the embarrassment that patients may experience with an organoleptic evaluation. Moreover, its simplicity makes it suitable for any type of consultation without requiring the training and calibration of an odour judge. In summary, the new test is able to detect amines efficiently in saliva. The results of the enzymatic reaction can be interpreted by using a simple colour scale. The level of detected amines clearly differs in patients with and without oral malodour. The performance of the test appears to be betterthan other salivary colour tests and similar to OralChromaTM, one of the most used adjunct tools in the diagnosis of oral malodour.

Based on these results, it has been concluded that regardless of the inability of amines to contribute

directly to the odour of saliva, they can be used as an indicator for bad breath. The new test can be considered as a simple, sensitive and objective adjunct tool for the clinical diagnosis of oral malodour [67].

Finally, a DAO from *Lathyrus sativus* has been used as a biocatalytic component of an electrochemical biosensor for the determination of the biogenic amine index in wine and beer samples. DAO from *L. sativus* was amperometrically characterized, and the obtained results allowed to compare the enzyme to other amine oxidases and to evaluate its selectivity towards different typical biogenic amines. The DAO was immobilized and employed to develop a novel DAO-based amperometric biosensor; the latter was analytically characterized, using putrescine as standard, under flow injection conditions. In order to reduce the influence of a matrix effect, which could affect the detection of biogenic amines in real samples, a special SPE, employing two working electrodes, was used as transducer. This dual-working electrode SPE, coupled to the proper multimeter (μStat 400 bipotentiostat, Dropsens, Oviedo, Spain) and driven by the DropView™ Software, allowed the simultaneous registration of both sample and blank signals and eventually to subtract the latter on the fly. The following analytical parameters were obtained using the dual-Au-SPE and putrescine as substrate: sensitivity 11.2±0.4 nA mg 1l, linear range 0.7–20.0 mg l-1 and LOD 0.2 mg l-1. The biosensor has been used for ten consecutive calibration plots without a significant loss of performance for the first five, confirming a good reproducibility, taking into account that it had been obtained using a screen-printed disposable transducer. In order to reduce any kind of interference, the analysis of real samples was carried out by a differential method employing the dual SPE-based biosensor described above. The measurements were carried out on two white wines, two red wines and two blonde beers. The registered amperometric signals were referred to that of putrescine, thus obtaining the total amount of BAs, considering that the developed biosensor displayed the highest catalytic efficiency towards putrescine. Biosensor's performances were validated using a modified reference method based on GC-MS analysis concluding that the biosensors accurately measure the overall BAs content expressed as putrescine equivalent in both red and white wines, being less efficient in beer samples where they measured only about 50% of the BAs content. The fast response, minimal sample treatment, and high sensitivity of the apparatus indicate that it may conveniently be employed for production plants or in wineries in order to provide a reliable estimate of the content of biogenic amines, a parameter that is being increasingly demanded by food quality commissions [68].

Inflammatory bowel diseases

Crohn's disease (CD) and ulcerative colitis (UC) are non-specific, non-infectious inflammatory bowel diseases (IBD) of the small and large intestine. They share several symptoms such as severe diarrhea, abdominal pain, vomiting, rectal bleeding, and high fever. However, while CD can affect any area of the gastrointestinal tract and is a deep destructive inflammation with a patchy and transmural tissue damage resulting in fibrosis and stenosis of the bowel, UC is confined to the rectum or to the distal part of the colon, and the ulceration does not go beyond the mucosa. Hereditary, immunologic and environmental factors are thought to be involved in the pathogenesis of IBD. According to accepted view, the disease develops in genetically predisposed hosts as a consequence of a disregulated immune response to enteric antigens resulting in continuous immune mediated inflammation and tissue damage. In CD, the responding T cells exhibit Th1, while in UC, Th2-phenotype lymphocytes are frequently found [69, 70]. In brief, Th1 lymphocytes secrete mainly IL-2, IFNγ and TNFα and are implicated in cell-mediated immunity, whereas Th2 cells secrete

IL-4, IL-5 and IL-13 and are related to strong antibody responses [71, 72]. Recently, two more pro-inflammatory interleukins, IL-17A and IL-17F, were found to be abundantly present in intestines of patients with UC and CD. These cytokines are products of newly disclosed and characterised Th17 lymphocytes. Helper Th17 cells produce all together six interleukins IL-17A-F, but beyond that, also IL-21, IL-22 and IL-9 are produced. They link innate and adaptive immunity mediated mucosal host responses to a number of gastrointestinal pathogens [73].

During the last few years, the incidence of IBD, quite high in industrialised Western countries, was more or less stabilized, but simultaneously, an increasing incidence rate has been observed in Finland, Eastern Europe and Asia [74]. Moreover, it has been reported that people who emigrate to Western countries have a higher risk for developing IBD, especially UC [75]. Noteworthy, UC and CD remain still incurable, despite considerable progress in the disclosure of underlying pathogenic mechanisms based on ongoing extensive clinical and experimental studies with various animal IBD models Table **1**.

Temporary remissions in IBD are followed by exacerbations of symptoms. Lately, the classic therapeutic approach, *i.e.* step-up therapy in relation to increased severity of IBD, is being confronted with a top-down strategy that involves early use of newly implemented immunomodulators and biological drugs Table **2**. Opponents to these therapies indicate side effects of the latter, such as increased risk of infection, hepatitis, pancreatitis, demyelinating disorders and malignancy [76-79]. On the other hand, persistent chronic inflammation is known to be itself strongly associated with increased tumour incidence and progression. Patients with UC or CD have a 2-3 fold greater risk of developing colorectal cancer than the general population [80]. The proponents of the top-down strategy stress that immune suppressants and biologics efficiently induce mucosal healing and maintain remission in CD patients, thus preventing irreversible damage of the bowel if used early, at onset of the disease [81, 82]. Thiopurines protect IBD patients against the development of advanced neoplasms [83]. However, in Caucasian patients these drugs increase a risk of non-melanoma skin cancer [84]. Arguments pro- and against top-down therapy in specific subgroups of IBD patients are reported in comprehensive reviews [82, 85, 86].

Without doubt, new biologic therapies offer treatment possibilities to some patients otherwise without chances for cure with conventional drugs. However, up to 50% of IBD patients are refractory to anti-cytokine compounds, or their positive response can cease with time. Patients treated with anti-TNF may experience severe side effects and extra-intestinal immune-mediated pathologies. Beyond that, these therapies are relatively expensive and have a narrow range of safety [81-83, 85, 86]. Therefore, the development of new strategies for combating the disease is a real challenge for IBD research. Anti-TNF therapy made it clear that apart the excessive production of proinflammatory cytokines, there is a defect in the function of regulatory principles in IBD.

In this respect, DAO may serve as a good approach. DAO is responsible for the catabolism of histamine and putrescine, both, and especially the latter, being intimately related to growth processes [87]. Histamine, widely distributed throughout the body, fulfills several important functions in the digestive tract: it stimulates secretion of gastric acid, other fluids and mucous, influences gut motility, stimulates growth and regeneration processes, increases collagen formation, participates in immediate allergic and inflammatory responses [88]. In healthy individuals, the enterochromaffin-like cells, mast cells and intramural nerves synthesize and supply histamine. In IBD individuals, mast cells hyperplasia is seen in inflamed mucosa and submucosa [89]. Besides the enhanced mastocytes, some other cells, including the infiltrating cells

(macrophages, lymphocytes), contribute to produce or promote synthesis of histamine. Histidine decarboxylase can be induced by TNFα, IL-1, GM-CSF [90]. This induced or so-called “nascent” histamine, acts on specific targets and, upon signaling, undergoes immediate degradation; it is not stored. However, in IBD not only the number of mast cells is increased, but their releasability, too. Thus, higher amounts of secreted histamine and other mediators, can be measured both *in vivo* [91, 92] or *ex vivo*, upon stimulation [93]. Histamine exerts a vast array of effects, interacting with four different G-protein coupled membrane receptors [94]. For example, via H_1 receptors, histamine evokes smooth muscle or endothelial cell contractions [95]. The same receptors also participate in increased vascular permeability and antibody production [96]. In turn, cytokine production, chemotaxis of mast cells and leukocytes (neutrophils, eosinophils), influx of calcium into these cells, which all define severity of inflammation, are mediated by H_2 and H_4 receptors [97, 98]. Histamine influences the Th_1 / Th_2 lymphocyte balance, affecting selectively cytokine production [99]. As becomes evident in UC: a shift towards Th_2, which it facilitates, results in more intensive inflammation and higher susceptibility to opportunistic infections and tumours. Histamine, through H_2 receptors, also induces plateled activating factor (PAF) production, increases adhesion molecules and can augment MHC class II antigen expression, stimulating the influx of immune cells into the intestinal wall. As already pointed out, histamine may also play a major role in the growth of normal and malignant tissue as a regulator of proliferation and angiogenesis [87, 99].

Histamine action is terminated essentially by two catabolic enzymes mentioned above: DAO and histamine N-methyltransferase (HMT). DAO is abundantly present in the vertebrate intestine showing characteristic activity profiles along the digestive tract, and across the intestinal mucosa. A low, variable activity of DAO in the stomach is followed by a gradual increase of its activity through the jejunum reaching a peak in the ileum. Its activity decreases towards the large intestine. Within the mucosa, the enzyme is present only in mature cells in the middle and in the tip of the villi. No DAO activity can be found in dividing crypt cells [1, 87]. Any disease involving intestinal mucosa damage seems associated with some loss of DAO activity. Indeed, in Crohn's ileitis, DAO activity is reduced [100, 101] and the decrease ofactivity is correlated to the severity of mucosal damage. The disease recurrence is preceded by a significant drop in DAO activity, making the tissue enzyme assay useful for prediction of the risk of recurrence or of anastomotic complications after resection [101]. DAO deficiency was claimed to be responsible for abnormal intestinal motility observed in CD patients. Small bowel circular muscle from CD patients demonstrated in *ex vivo* assays a much enhanced contractile response to histamine, blocked by H_1 receptor antagonists, but not modified by inhibitors of DAO [102]. In active UC, the decrease of DAO activity was much more pronounced than in CD; roughly 10% of normal DAO activity could be observed. However, during the remission phase, an over 5 fold increase above the regular activity was found and an antiproliferative rebound effect in these subjects was suggested [103]. Adaptive increase in the methylation pathway of histamine catabolism to compensate for the DAO deficit was reported [104]. Urinary methylhistamine output, significantly higher in acute phases of IBD, showed a correlation with the intensity of inflammation [104]. CD patients have physiological tissue histamine concentrations, while tissue histamine concentrations in UC patients are higher, suggesting a marked unbalance between histamine synthesis and its degradation [105].

Some evidence was published in the nineties, indicating that excess of histamine plays an important role in the symptomatology of the disease. Namely, ketotifen H_1 antagonist, with some mast cell stabilizing potency, was reported to block eicosanoid accumulation by cultured colonic mucosa from UC patients [106].

Ebastine, another H_1 blocker, was able to inhibit T cell migration and production of Th_2 type cytokines by cultured lymphocytes and production of proinflammatory cytokines by macrophages isolated from healthy donors [107]. Interestingly, in a small group of children with moderate UC, an improvement was noted with ketotifen treatment [108]. More recently, this problem was addressed in rat models of UC using histamine receptor antagonistic ligands. Histamine H_1 (ketotifen), H_2 (ranitidine) and dual H_3/H_4 (thioperamide) receptor ligands were administered immediately after UC induction and treatment was continued for 5 consecutive days [55]. In another study on rat UC model, two selective antagonists of H_4 receptor (JNJ 10191584 and JNJ 7777120) were used [109]. Both studies have shown that interference with histamine signaling at the level of its receptors resulted in improvement of animal health; parallelly, the markers of inflammation as well as mucosal inflammation scores were lower. Benefits were most probably due to inhibition of histamine interaction with various components of the immunological system [96]. Recent work suggests that in the digestive system of patients with IBD, there is an up-regulation of H_1 and H_2 receptors [95].

The second strategy was based on the idea that if the disease results from a lack of regulatory principle, therapy should deliver it. Therefore, DAO (a commercially available preparation from dog kidney, immobilized on Concanavalin A Sepharose) was administered via intraperitoneal route to rats immediately after intrarectal administration of 4% acetic acid for induction of intestinal injury and colitis. The 5 days therapy improved plasma ceruloplasmin and myeloperoxidase activity in the large bowel, colon injury, and inflammation scores. The colonic DAO activity in the treated rats was significantly higher, indicating enzyme binding by the tissue.

These results are very promising and suggest that appropriately prepared DAO formulations could be implemented in antiallergic and anti-inflammatory therapy. The remplacement of pancreatic enzymes has saved many lifes. DAO may prove equally successful in the future.

Fig. (1). Mechanisms involved in the pathogenesis of intestinal ischemia/reperfusion injury: role of histaminases.

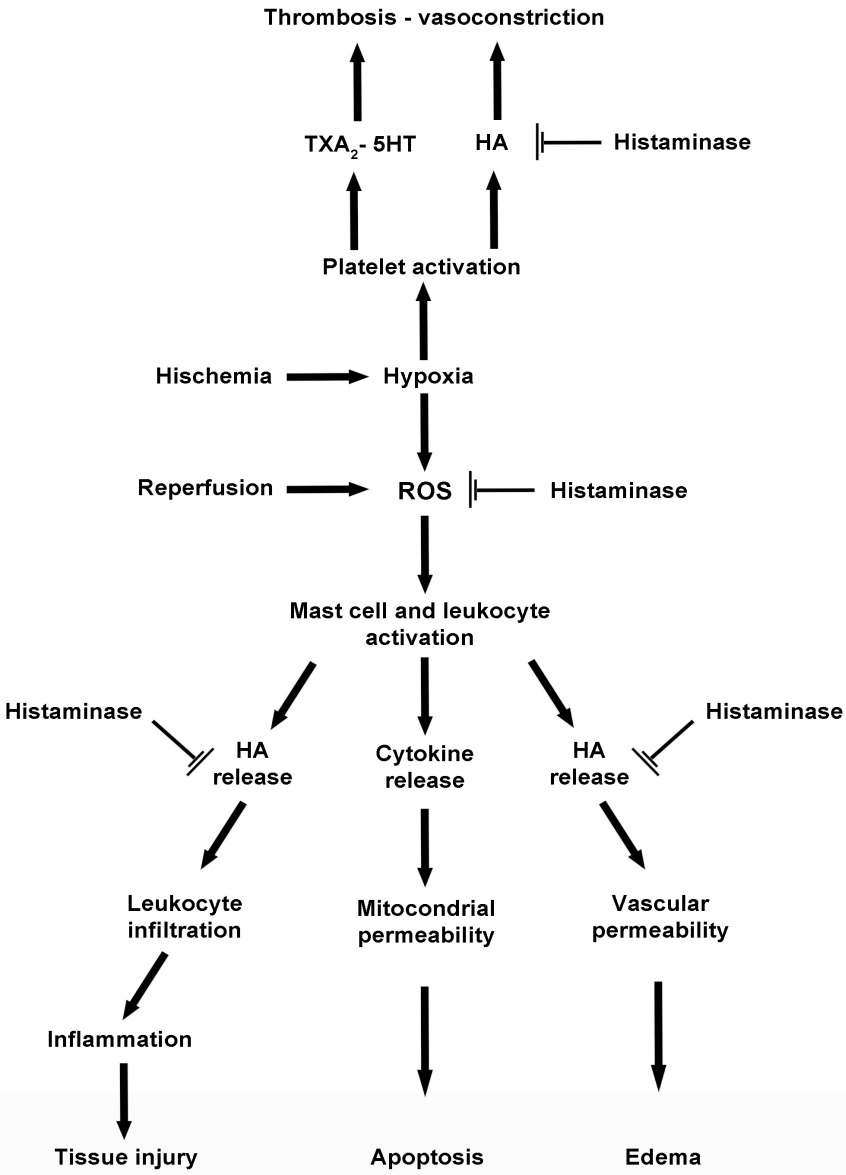
Thrombosis - vasoconstriction
TXA2- 5HT
HA
Histaminase
Platelet activation
Hischemia
Hypoxia
Reperfusion
ROS
Histaminase
Mast cell and leukocyte activation
Histaminase
HA release
Cytokine release
HA release
Histaminase
Leukocyte infiltration
Mitocondrial permeability
Vascular permeability
Inflammation
Tissue injury
Apoptosis
Edema

Table 1. Some more common IBD animal models [110-114].

IBD Type	Agents
Chemically induced	Acetic acid Iodoacetamide 2,4,6-Trinitrobenzene sulfonic acid (TNBS) Oxazolone Dextran sulfate sodium
Adoptive transfer models	Heat shock protein (hsp) 60- specific CD8 T cells $CD4^+$ $CD45RB^{hi}$
Gene knockout (KO) models	Interleukin-2 KO/IL-2 receptor (R)α KO IL-10 KO T cell receptor (TCR) mutant Trefoil factor-deficiency TNF-3' untranslated region (UTR) KO
Transgenic animal models	Signal transducer and activating transcription-4 (STAT-4) IL-7 transgenic HLA B27 transgenic
Spontaneous Colitis	C3H/HejBir mice SAMP1/Yit mice

Table 2. Multiple treatment options for IBD [115-120]

Synthetic drugs	5- Aminosalicylates (mesalazines), glucocorticosteroids, Azathioprine, 6-mercaptopurine Methotrexate, cyclosporin A
Biological therapeutic agents	Proinflammatory cytokine inhibitors: anti-TNFα, anti-IL12/ 23, Anti-inflammatory cytokines: RHIL-10, RHIL-11 Anti-integrins

Patent review coverage on the treatment of allergy and inflammation

Treatments based on inhibiting mast cell degranulation and histamine release

Inflammatory bowel diseases, such as UC and CD, present a chronic and relapsing intestinal inflammatory status that is principally caused by immune deregulation of the gut. Despite significant advances in therapeutic strategies (including corticosteroids, 5-aminosalicylates, immunomodulators), there is no efficacious method to treat IBD. As the inflammatory-mediated affections are complex and multi-factorial, different strategies for prevention and treatment of inflammation have been proposed in various patent applications.

New active molecules, such as novel pyrimidine derivatives, formulated as tablets, capsules, powders, solutions or suspensions, were reported for the treatment of IBD and of immunological diseases

[121]. Other patent applications are based on some chemical compounds (bicyclic heteroaryl-substituted imidazoles, benzofuro- and benzothienopyrimidine, 2-aminopyrimidine) as modulators of histamine receptor activity (H_4R) exposed on eosinophils, basophils and mast cells, for treatment of different pathological conditions including allergy [122-124]. The release of inflammatory mediators is thus well controlled in the presence of these H_4R modulators, blocking the leukocyte recruitment and hindering the inflammation process. Various suitable routes of delivery of these modulators are also proposed (*e.g.* oral, rectal, topical).

For patients having a medical condition related to the histamine H_1 receptor (H_1R), such as allergy and inflammatory diseases, a RNA_i-mediated inhibition of H_1R was proposed [125]. The administration of RNA_i silences the expression of the H_1R, preventing the events occurring in histamine-mediated inflammatory responses.

The inhibition of the binding of a histamine releasing factor to the immunoglobulin E (IgE) was reported to treat food allergy, allergic reactions or inflammatory reactions [126]. It is known that the histamine releasing factor contributes to the stimulation of the histamine release and to the production of IL-4 and IL-13 cytokines from IgE-sensitized basophiles. Thioredoxin, a small dithiol protein, can also have a positive effect in the food allergy treatment, decreasing the allergenicity of food allergens, as reported in animal experiments [127].

Other bioactive compounds that can inhibit mast cell degranulation could also be of interest. Thus, various peptides were reported for the treatment of cutaneous inflammation (dermatitis, eczema, psoriasis) and of different allergies (food sensitivity, anaphylaxis), being potentially useful to treat also IBD by inhibiting mast cell degranulation, and, thus, reducing the release of histamine, cytokines and leukotrienes [128, 129]. In this context, these peptides could be successfully used to replace corticosteroids.

A pharmaceutical composition containing a therapeutically effective amount of a naturally-derived isoorientin (extracts of aloe, rice plant, bamboo, etc), as an anti-histamine active ingredient, was reported by Woo *et al.* [130] for the prevention or treatment of different conditions associated to an excessive quantity of histamine. The important anti-histamine effect of isoorientin is based on the inhibition of histamine release from mast cells in a concentration-dependent way. Such compositions, administrated orally or parenterally, may be useful for the prevention and the treatment of allergy or inflammatory diseases, where histamine plays a major role.

A crude vegetal extract based on a hardy kiwifruit (*Actinidia sp.*) was reported to prevent and to treat allergy and non-allergic inflammatory diseases (different forms of dermatitis, gastritis, enteritis, etc), principally, by inhibiting histamine release from mast cells and by reducing the level of Th_2 cytokines and IgE in serum [131, 132]. The vegetal extract can be formulated as tablet, capsule, powder or suspension [131]. Food supplements or pharmaceutical compositions based on plant extracts of *Momordica sp.* (*Momordica charantia*, *Momordica dioica*) [133], of *Tiarella polyphylla* [134] or of *Allium vineale* [135], formulated for systemic, oral or topical / transdermal administration, can also be used as pharmacologically active compounds for the treatment of conditions or diseases associated with the release of histamine (food allergy, drug allergy, anaphylaxis). The extract of *Tiarella polyphylla* (containing tiarellic acid) was reported to exert suppressive effects on the production of IgE and of some cytokines (IL-4, IL-5, IL-13), and on leukocyte infiltration, being proposed as food supplement or as therapeutic agent to prevent or to treat the pathological conditions mentioned above [134].

A herbal therapy, consisting of a mixture of Chinese herbs, such as FAHF-1/2 herbal formula, was

reported by Xiu-Min and Sampson [136] for the treatment of food allergy, reducing mast cell degranulation and histamine release, lymphocyte proliferation and synthesis of IL-4, IL-5 and IL-13. This herbal therapy can be administered with a pharmaceutically adequate carrier, adjuvant or vehicle (orally, parenterally, intraperitoneally, topically, etc), and used in combination with other active agents, such as anti-histamines. Compared to standard immunosuppressive therapy, generally using corticosteroids, it seems that this herbal therapy is more selective for the allergic response.

Other compositions (comprising luteolin from *Perilla* leaf or seed, Cinnamon, Kiwi, Hesperidin etc), which prevent or inhibit the release of histamine, prostaglandin D_2 and/or leukotriene C_4, and thus reduce or stop allergic or inflammatory responses, were also reported [137].

Other proposed therapies for inflammation

Novel bioactive polyamine analogs and their derivatives, used to inhibit the expression or activity of TNFα and/or different cytokines (IL-2, IL-6, IL-8, IL-12, etc) that usually are expressed in inflammatory diseases, have been reported [138]. These bioactive agents can be used in combination with other active anti-inflammatory agents. An anti-inflammatory therapeutic composition, principally based on *γ*-tocopherol and/or on esters of *gamma*-tocoferol, was reported to reduce inflammation in IBD by diminishing the expression of endothelial cell adhesion molecules in the vasculature of the bowel wall, additionally to the antioxidant use of *γ*-tocoferol [139].

Amine oxidases in treatment of allergy and other histamine–mediated conditions

Oxygen-free radicals are known as pro-inflammatory agents. Wu and McIntire [140] postulated a new treatment for pathologies associated with oxidative damage, providing compositions comprising at least one recombinant peroxisomal polyamine oxidase (PAO) to treat inflammation or other pathological conditions: for exemple PAO as *antioxidant* in ischemia/reperfusion and as *pro-oxidant* in cancers (due to the released H_2O_2). Thus, the invention relates to intracellular polyamine dysregulation, as it was observed that some elevated levels of polyamines are associated with reduced PAO activity. Pharmaceutical compositions comprising recombinant PAO may be administered either systemically or locally.

AOs were reported as useful biosensors for the detection of histamine in food and beverages, as mono-enzymatic system (AO) or bi-enzymatic system (AO and peroxidase) [141]. Based on the same principle of histamine degradation by AOs, a similar approach to degrade histamine was introduced by Missbichler *et al.* [142]. This recent patent application reported pharmaceutical compositions containing a DAO of animal origin (DAO from pig kidney or recombinant DAO prepared in cell cultures) for the treatment and/or prevention of histamine-induced conditions [142]. The composition containing DAO can be formulated for epidermal, oral or sublingual administration. For the oral administration of DAO, gastric-resistant pellets, coated with Eudragit or Shellac, and gelatine or starch capsules were used.

Proposed innovative treatment of allergy and inflammation and its relationship with the reported patent applications

A new concept for the treatment of different conditions, such as allergy, CD and UC, based on the oral administration of a vegetal DAO (VDAO) associated to catalase, was recently disclosed [143]. An oral bi-enzymatic therapy with VDAO and catalase was suggested to reduce the levels of intestinal histamine via

degradation by DAO, with the production of H_2O_2, NH_3 and imidazole acetaldehyde. As H_2O_2, a by-product of degradation, is toxic, having pro-oxidant effects on intestinal cells, catalase was proposed, as second therapeutic enzyme, to decompose H_2O_2. The association of catalase and DAO has two major beneficial effects: first, catalase will prevent the local intestinal oxidative stress by decomposing H_2O_2 produced by VDAO, and, secondly, by decomposing H_2O_2, catalase will generate oxygen, a substrate for DAO. This may contribute to the enhancement of histamine oxidation and to a shift of equilibrium of the DAO enzymatic reaction towards the reaction products. Since the availability of oxygen in the colon is limited, oxygen produced by the histaminase reaction will probably be beneficial for the allergy or IBD treatment, by more efficient degradation of intestinal histamine [144].

As already shown by Aschenbach *et al.* [145], an Organic Cation Transporter (OCT), located at the level of intestinal junctions, contributes to the transport of histamine from the intestinal lumen into systemic circulation. The same OCT also facilitates the transport of histamine from circulation into the intestinal lumen (bio-elimination), with the risk of readsorption Fig. (**2**). Our new concept of treatment by oral administration of VDAO associated to catalase, will prevent the readsorption of histamine into the systemic circulation, will enhance the intestinal bio-elimination of histamine and will eliminate the toxicity of H_2O_2. Catalase will also protect DAO from H_2O_2 produced by DAO itself (in the presence of histamine) or by other inflammatory reactions.

The design of oral delivery systems protecting sensible therapeutic agents such as VDAO and catalase [143] against digestive enzyme degradation, represents a major challenge. By targeting the intestinal sites, these therapeutic enzymes must remain active to exert their therapeutical action. In this context, VDAO and catalase were formulated as monolithic tablets based on carboxymethyl starch (able to protect the enzyme against gastric acidity) associated with chitosan in order to deliver these bioactive agents to the colon [143, 144]. Other pharmaceutical excipients (*i.e.* metacrylates, chitosan, carboxymethyl starch or their mixtures) can also be used.

As already mentioned above, histamine is an important mediator in several pathological conditions. The majority of the patent applications reported here are focused on different strategies to reduce or to inhibit the release of histamine by: a) modulation of histamine receptors, administering various synthetic molecules [121-124] or RNA_i that silences the expression of histamine receptors [125], b) inhibition of the binding of a histamine releasing factor to IgE [126], c) suppression of IgE production [134], d) decrease of the allergenicity of food allergens by reducing the allergenic proteins containing disulfide bonds to the sulfhydryl (SH) level [127], e) reducing or inhibition of mast cell degranulation, administering different active agents, such as peptides [128, 129], naturally-derived isoorientin [130], vegetal extracts of *Actinidia sp.* [131, 132] or other vegetal extracts [136].

The proposed innovative therapies of Mondovi *et al.* [24] and Mateescu *et al.* [143] are mainly focused on *histamine degradation* rather than on the reduction or inhibition of histamine release, such as reported in the majority of histamine–related patent applications here described. The new proposed therapy will act directly on histamine, degrading it before it exerts its effects. Thus, by using the proposed oral bi-enzymatic therapy, histamine - mediated affections could be reduced or even prevented. Other patent applications for the therapy of allergy and inflammation are focused on *histamine degradation* by administration of different AOs as bioactive agents [140, 142, 145-147].

The application of Mateescu *et al.* [143] is related to the patent of Mondovi *et al.* [146] that

proposed for the first time the use of a VDAO, formulated as spray and for topical applications, in the treatment of histamine - mediated conditions. However, this disclosure [143] differs essentially from the previous one [146], by proposing a novel oral bi-enzymatic treatment of histamine-mediated intestinal conditions: an *oral administration* of VDAO associated to catalase. Other patent applications describe the use of AOs produced by recombinant DNA techniques to treat intestinal immune or inflammatory disorders related to histamine [140, 147]. The use of a vegetal histaminase presents several advantages, such as: (i) higher enzymatic activity (30-50 times) than the animal enzyme, (ii) larger accessibility (extraction from different vegetal sources using a simple procedure), (iii) less expensive regarding production of large quantities, (iv) better acceptability of a vegetal agent by regulatory organisations.

Oral administration of DAO in pharmaceutical compositions or as food supplements, used to degrade histamine at intestinal level, was mentioned for the first time in the patent application of Missbichler *et al.* [142]. The use of a non-vegetal DAO was preferred in this application due to the possible presence of allergens in vegetal extracts containing DAO.

It is known that low doses of H_2O_2 generated *in situ* can exert anti-inflammatory effects [148] whereas, high concentrations of H_2O_2 can produce nocive effects, affecting cell viability. The difference and the advantage of the Mateescu *et al.* [143] application resides in the possibility to eliminate *in situ* the produced H_2O_2 resulting from histamine degradation or from other inflammatory reactions, by associating catalase to VDAO. This novel bi-enzymatic therapy proposes, first, to degrade histamine from the gut in the presence of VDAO (preventing its return into circulation), and, secondly, to decompose H_2O_2, the by-product of VDAO, by catalase. Furthermore, as reported for other copper oxidases [149-151], VDAO presents antioxidant properties, that complete its histaminase action. The antioxidant capacity of VDAO, counteracting the free radical species released at intestinal level, would be beneficial in the treatment of histamine-mediated conditions. This novel approach of an oral bi-enzymatic formulation of VDAO and catalase will be of high utility and will also open a new concept of biopharmaceutically assisted clearance for various harmful biomolecules, particularly in systems where the toxicants (*i.e.* histamine or H_2O_2 by-product) are substrates for orally administered enzymes (*i.e.* VDAO and catalase).

CURRENT & FUTURE DEVELOPMENTS

The majority of the patent applications reported here are focused on various strategies to reduce or to inhibit the re- lease of histamine (i.e., modulation of histamine receptors, suppression of IgE production, inhibition of mast cell de- granulation, etc.). Different from these strategies, the innovative therapies discussed in this review are mainly focused on histamine degradation rather than on the reduction or inhibition of histamine release. Thus, a natural enzymatic therapy of histamine-mediated diseases consisting of the administra- tion of a plant AO may open the way to alternative non-toxic and innovative therapeutic approaches for the control of dif- ferent histamine-mediated pathological conditions, such as allergy, anaphylaxis, intestinal ischemia, IBD. The plant AO will naturally decrease the level of harmful histamine, known to participate in the pathogenesis of the affections mentioned above. As the H2O2 by-product of AO is a prooxidant agent with some undesirable oxidative damaging effects, a bi-enzymatic treatment consisting of the association of the plant AO (DAO) with catalase, an enzyme de-composing H2O2, was proposed [138, 139]. In addition to its histaminase action, the vegetal DAO will probably exert antioxidative effects, such as previously described for other copper oxidases (i.e., ceruloplasmin, bovine serum amine oxidase). Since the oxidative

stress is involved in different histamine-related pathogenesis, in the future, it will be of interest to have a better understanding of the additional anti- oxidant properties of plant AOs.

Different approaches of plant AOs forms of administra- tion are presented here (i.e., systemic, oral administration). Various strategies for targeting plant AOs release selectively to different sites have to be developed in the future. A challenging way will be the oral route to administer AO in order to control the histamine-mediated pathological conditions at intestinal level. These novel therapies for the histamine-related pathologies have to be further proven in experimental models of the disease in animals and also in humans.

An important aspect is the acceptability of natural vegetal therapeutic protein for oral administration. The AOs also present a potential role in tumor therapy. When formulated with excipients as pharmaceutical forms for colon delivery, they can be used for intestinal cancer therapy in association or not with other antitumor chemotherapeutic drugs.

In conclusion, the recent advances in understanding the beneficial actions of plant AOs in histamine-mediated affec- tions make plant AO an interesting biopharmaceutical molecule. Different from the current proposed therapies of the histamine-mediated conditions mentioned above, the admini- stration of a plant AO could represent a safe and non-toxic alternative that can completely replace or act complementary.

with other existing treatments. Thus, natural treatments will ensure patient compliance, better patient health and im- proved quality of life.

Abbreviations

AO: Amine Oxidase

BzAO: Benzylamine Oxidase

CuAO: Copper Amine Oxidase

DAO: Diamine Oxidase

HMT: Histamine N-Methyl Transferase

IBD: Inflammatory Bowel Diseases

ICAM: Intracellular Adhesion Molecule

LoVoWt: LoVo Wild type

MAO B: Monoamine Oxidase B

NO: Nitric Oxide

NOS: Nitric Oxide Synthase

OCT: Organic Cation Transporter

PAF: Platelet Activating Factor

PAO: Polyamine Oxidase

ROS: Reactive Oxygen Species

SAO/R: Splanchnic Artery Occlusion and Reperfusion

SSAO: Semicarbazide Sensitive Amine Oxidase

TPQ: 2,4,5-TrihydroxyPhenylalanineQuinone

UC: Ulcerative Colitis

VDAO: Vegetal DAO

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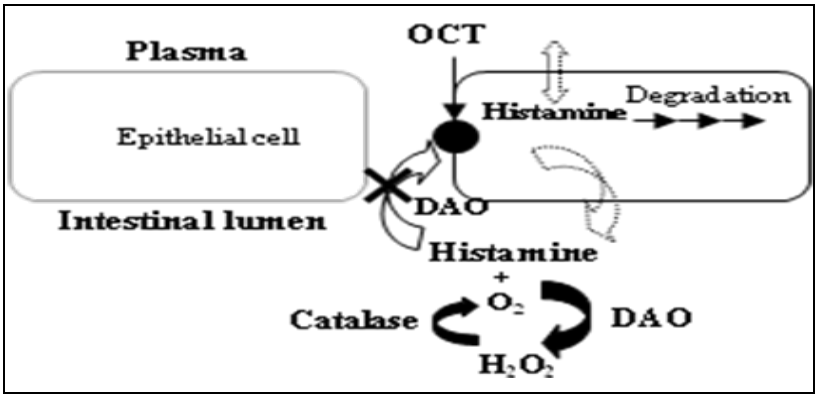
CONFLICT OF INTEREST

The authors declare that they don't have any conflict of interest and that they have received no payment for writing this review.

Fig. (2). Model depicting intestinal elimination of histamine
DAO: Diamine Oxidase; OTC: Organic Cation Receptor.



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