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Evaluation of the inhibitory effect of various drugs / active ingredients on the activity of human diamine oxidase *in vitro*

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Introduction

Diamine Oxidase (DAO; EC 1.4.3.22) is the most significant enzyme in the degradation of biogenic amines in the intestine. Ingestion of foods high in biogenic amines (e.g. Histamine), together with a reduced DAO activity leads to accumulation of histamine, which in turn can trigger symptoms of histamine intolerance / biogenic amine intolerance syndrome (BAIS). A multiplicity of pharmaceuticals have been suggested to influence DAO activity, however so far few experimental data were available to support this hypothesis. This present study assayed both active ingredients and factory-made pharmaceuticals for possible DAO activity inhibition.

Relevance

DAO inhibition can occur in acute or chronic inflammation of the intestinal mucosa [1], as well as after alcohol consumption. Other biogenic amines such as putrescine and cadaverine can compromise DAO activity [2].

For some pharmaceuticals a DAO inhibition was assayed in vivo using animals, however most records on DAO inhibition were reported in patients [3,4].

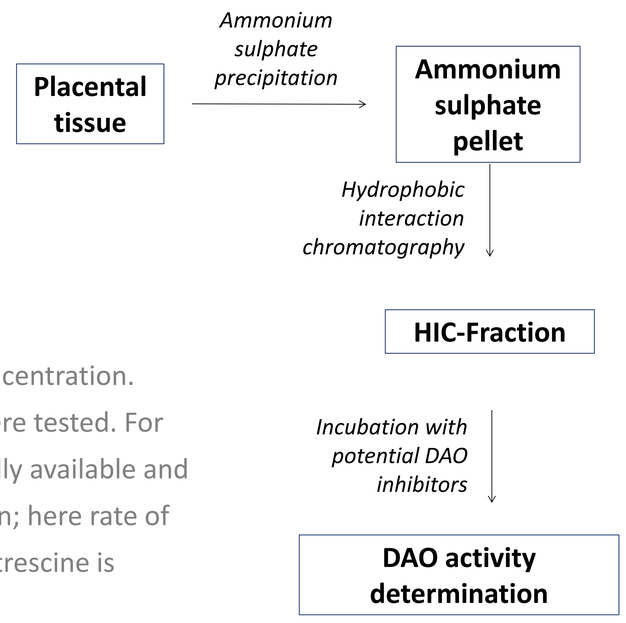
Methods

A selection of drugs as well as their corresponding active ingredients was compiled according to the medical literature. Interaction between chromatographically purified DAO was determined using an enzyme activity assay. Various drugs were incubated with the enzyme in the recommended pharmacological concentrations.

Enzyme inhibition was calculated referring to the control (no inhibitor present). To test the influence of the excipients both API and whole dosage form were assayed.

Experimental Setup

Human placental tissue was minced and subjected to a fractionated ammonium sulphate precipitation (35% / 65%). A fraction high in DAO activity was purified using hydrophobic interaction chromatography (HIC), and incubated with the drugs in prescribed concentration. Both API and commercial dosage forms were tested. For determination of DAO activity a commerially available and validadet radio extraction assay was chosen; here rate of conversion of Tritium-labelled [1,4- ^{3}H]-putrescine is determined.



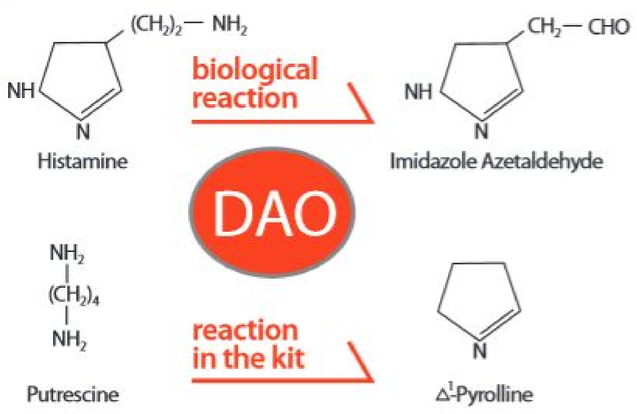


Fig. 1: Diagram of the radio extraction assay:
Under physiological conditions diamine oxidase converts histamine to imidazole acetaldehyde. In this assay, hydrophilic [1,4- ^{3}H] putrescin is converted to hydrophobic Δ^{1}-Pyrroline, which can be extracted using organic solvents and assayed via liquid scintillation counting

References:

[1] Raithel, M., et al., Mucosal histamine content and histamine secretion in Crohn's disease, ulcerative colitis and allergic enteropathy. Int Arch Allergy Immunol, 1995. 108(2): p. 127-33.

[2] Missbichler, A., A. Bayer, and R. Leitner, Investigating the Biochemical Background for Histamine Intolerance: Degradation Capacity of Mixtures of Biogenic Amines by Human, Porcine and Bovine Diamine Oxidase. Ann Nutr Metab, 2012. 61(4): p. 330

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[5] I. Mayer, A. Missbichler, F. Wantke, M. Focke, H. Reichl, M. Winter und R. Jarisch: Optimierter Radioextraktionsassay zur quantitativen Bestimmung der Aktivität von Diaminooxidase (DAO) in humanem Serum und Plasma Allergologie, 28, Nr. 1/2005, pp. 1–8

Results

Quality of the chromatographic purification of DAO was determined assaying 1 ml aliquots via DAO activity assay.

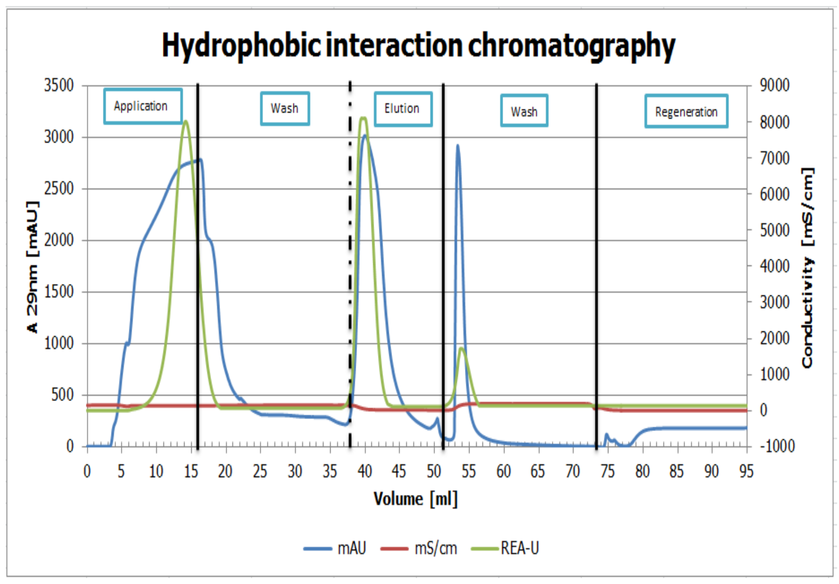

Fig. 2: Hydrophobic interaction chromatography using an ÄKTA Explorer system (GE-Healthcare).

Process segmented into: equilibration, application of protein, wash, elution of target protein, wash, regeneration of column.

x-axis:
Volume of mobile phase [ml]
primary y-axis:
absorption at λ_{max}=290nm [mAU]
secondary y-axis:
conductivity [mS/cm]
tertiary y-axis:
enzyme activity [REA-Units]

DAO-rich fractions were incubated with ^{3}H-Putrescin plus the appropriate API resp. composite drug dosage form. Total active ingredient concentration was chosen to reflect the duodenal/jejunal concentration (site of maximal physiological DAO concentration) if taken in the recommended dosage.

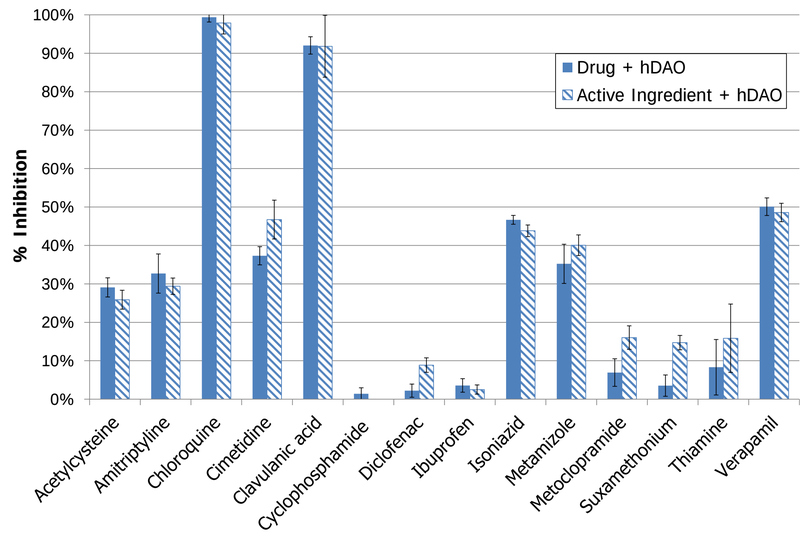

Fig. 3: % inhibition of DAO activity by drug and API in recommended dosage.

Calcuation of inhibition was done relative to the enzyme activity without inhibition minus non-specific binding (NSB)

$$\left(1-\frac{sample-\ NSB}{control-\ NSB}\right)*100 = \%\ Inhibition$$

Chloroquine and Clavulanic acid inhibit DAO activity practically completely (> 90%).

Isoniazid und Verapamil show modest DAO-inhibition of about 50% . Also modest DAO-inhibition >20% was found with Cimetidine, Metamizol, Acetyl cysteine und Amitriptyline.

Metoclopramide and Thiamine inhibit DAO only weakly (<10%).

Ibuprofen, Suxamethonium chloride,Diclofenac and Cyclophosphamide and showed no effect on DAO activity (<5% inhibition)

	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

Fig. 4: Summery of tested substances

Conclusion

Considering a DAO inhibition of more than 30% critical, most of the tested drugs can be designated DAO inhibitors. Excipients in the pharmaceutical dosage forms (capsules or tablets) did not influence DAO activity. Thus it would be advisable to inform patients with diagnosed histamine intolerance / biogenic amine intolerance syndrome about possible side effects of these drugs.

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