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 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 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].

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 commercially available and validated radio extraction assay was chosen; here rate of conversion of Triterium-labelled [1,4-3H]-putrescine is determined.

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

Chloroquine and Clavulanic acid inhibit DAO activity practically completely (>90%).
Isoniazid and Verapamil show modest DAO-inhibition of about 50%. Also modest DAO-inhibition >20% was found with Cimetidine, Metamizol, Acetyl cysteine und Amitriptyline.
Metoprolacmide and Thiamine inhibit DAO only weakly (<10%).
Ibuprofen, Suxamethonium chloride, Diclofenac and Cyclophosphamide showed no effect on DAO activity (<5% inhibition)

Fig. 4: Summary of tested substances

DAO-rich fractions were incubated with 3H-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.

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.

References:

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

Fig. 2: Hydrophobic interaction chromatography using an ACE5 Explorer system (GE-Healthcare).
Process segmented into: equilibration, application of protein, wash, regeneration of column.

Fig. 3: % inhibition of DAO activity by drug and API in recommended dosage.
Calculation of inhibition was done relative to the enzyme activity without inhibition minus non-specific binding (NSB) at 100% inhibition.

Fig. 2: Hydrophobic interaction

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Inhibition</th>
<th>effect</th>
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<tbody>
<tr>
<td>strong DAO inhibitors</td>
<td>Chloroquine</td>
<td>99%</td>
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<tr>
<td>Clavulanic acid</td>
<td>93%</td>
<td>antibiotic</td>
</tr>
<tr>
<td>Metoclopramide</td>
<td>7%</td>
<td>antiemetic</td>
</tr>
<tr>
<td>Isoniazid</td>
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<td>antibiotic</td>
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<td>Metamizol</td>
<td>35%</td>
<td>analgesic/antipyretic</td>
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<td>Ascorbic acid</td>
<td>20%</td>
<td>mucolytic agent</td>
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<tr>
<td>Thiamine</td>
<td>8%</td>
<td>vitamin</td>
</tr>
<tr>
<td>Metoprolacmide</td>
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<td>antihypertensive</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>4%</td>
<td>NSAID</td>
</tr>
<tr>
<td>Suxamethonium chloride</td>
<td>4%</td>
<td>depolarizing neuromuscular blocker</td>
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<td>Clavulanic acid</td>
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<td>NSAID</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>1%</td>
<td>Chemotherapeutic</td>
</tr>
</tbody>
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Fig. 2: Hydrophobic interaction chromatography using an ACE5 Explorer system (GE-Healthcare).