Faecal tryptic activity in humans: influence of antibiotics on microbial intestinal degradation

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Soluble faecal tryptic activity (FTA) was determined in healthy adults using a spectrophotometric method. Mean FTA was 100 mg/kg faeces (range 0–980), inter-individual variations were more pronounced than the intra-individual ones. Ten different antimicrobial drugs given orally for six days caused alterations in some individual FTA levels.

KEY WORDS—Tryptic activity; germfree life; intestinal flora; antibiotics.

INTRODUCTION

Proteolytic activity in faeces represents a combination of activated pancreas derived pro-enzymes (variants of trypsinogen, chymotrypsinogen A and B and pro-elastase 1 and 2) in combination with microbial proteases, which are all under the influence of the host, and microbial and dietary derived activators and inactivators. The activated pancreas derived endopeptidases are necessary for a normal intestinal digestion of protein. Trypsin hydrolyses peptide bonds adjacent to arginine or lysine; chymotrypsin hydrolyses peptide bonds adjacent to tryosine, tryptophan or phenylalanine and elastase acts on the peptide bonds adjacent to serine, leucine, alanine or valine residues.

Erlanger et al. showed that the chromogenic substrate BAPNA (N-benzoyl-DL-arginine-paranitroanilide hydrochloride) was suitable for the assay of trypsin, and we have found this substance suitable when investigating tryptic activity in intestinal and faecal extracts from germ-free and conventional rats.

In a recent investigation by MacFarlane et al. it was shown that proteolytic activity in stools from five healthy humans, living on normal Western diets, varied between 3-5 and 19-8 mg of azocasein hydrolysed hourly/g faeces. The authors stressed the fact that ‘at present, few data are available concerning the extent and nature of protein degradation in the human colon’.

The aim of our study was to investigate the levels of faecal tryptic activity (FTA), using the BAPNA substrate, in healthy adult volunteers living on normal Western diets. Additionally, we wanted to evaluate whether ten different orally given antimicrobial drugs would influence the FTA levels. This investigation is one part of ongoing studies concerning microbial intestinal interactions on host-derived biochemical functions.

MATERIALS AND METHODS

Faecal samples

Faeces from 65 healthy volunteers of both sexes, median age 25 years (range 19–40) and median weight 61 kg (range 48–90), were investigated. None of the volunteers had experienced episodes of gastrointestinal disorder or infection that needed antibiotic therapy during the last three months prior to the first faecal sampling period, and all of them lived on normal Western diets. The volunteers gave informed consent to take part in the study which was performed in accordance with the revised Declaration of Helsinki, and the investigation was approved by the local ethical committee.
Table 1. Levels of faecal tryptic activity (FTA) in human samples.

<table>
<thead>
<tr>
<th>mg FTA per kg faeces</th>
<th>Individual FTA values before antibiotics</th>
<th>Difference between two samples from each individual within one week</th>
<th>Difference between samples obtained before and 5 weeks after antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>no activity</td>
<td>24 37 per cent</td>
<td>77 per cent—agreement</td>
<td>64 per cent—agreement</td>
</tr>
<tr>
<td>up to 100</td>
<td>22 34 per cent</td>
<td>15 per cent—minor discrepancies</td>
<td>22 per cent—minor discrepancies</td>
</tr>
<tr>
<td>100–199</td>
<td>7 11 per cent</td>
<td>5 per cent—major discrepancies</td>
<td>12 per cent—major discrepancies</td>
</tr>
<tr>
<td>200–399</td>
<td>9 14 per cent</td>
<td>3 per cent—very major discrepancies</td>
<td>3 per cent—very major discrepancies</td>
</tr>
<tr>
<td>over 400</td>
<td>3 5 per cent</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean 100 mg (kg faeces)⁻¹ (range 0–980).

Antibiotics

The volunteers were randomly divided into ten groups receiving the following antimicrobial drugs for six days; ampicillin 500 mg × 4, bacitracin 25 000 IU × 4, clindamycin 150 mg × 4, cotrimoxazole 160/800 mg × 2, doxycycline 200 mg on the first day followed by 100 mg per day, erythromycin 250 mg × 4, metronidazole 400 mg × 3, nalidixic acid 500 mg × 4, ofloxacin 200 mg × 2 and vancomycin 240 mg × 4.

Sampling

Faecal sampling was performed twice during the week prior to the respective drug administration period, on day six during intake and one and five weeks after completion of intake. The samples were frozen in plastic bags within 30 minutes and kept frozen until analysed. The soluble FTA fraction (the supernatant) was obtained after homogenizing faeces 1:2 with saline, and the resultant homogenate kept at 4°C for 2 h prior to centrifugation at 35 000 g for 30 minutes at 4°C.

Measurement of FTA

0.1 ml from each supernatant was added to 2.9 ml 0.1 M tris-buffer (pH 8.2, 4.4 g calcium chloride per litre), and the reaction initiated by adding 0.6 ml of 0.003 M BAPNA (Sigma, St. Louis, Mo. USA). The reaction was performed at 20°C for 10 min and stopped by adding 0.6 ml 5 M acetic acid. For the preparation of a standard curve bovine pancreas trypsin type 111 (Sigma) diluted in 2 mM hydrochloric acid was used. All samples were analysed in parallel with blanks from respective samples and the readings performed on a Hitachi 150–200 spectrophotometer at 405 nm. The amount of enzyme activity in the sample was calculated after correction for the blank value and expressed as mg FTA/kg faeces.

RESULTS AND DISCUSSION

The mean level of FTA was 100 mg/kg faeces (Table 1). One third of the samples were devoid of any FTA and in one third, the activity never exceeded 100 mg/kg faeces. Only 5 per cent of the samples contained more than 400 mg/kg faeces. When comparing samples taken at intervals of one and five weeks respectively, it was found that the inter-individual variations were more pronounced than the intra-individual ones.

The results in Table 1 demonstrate that presence of low values of FTA in faeces from healthy adults on normal Western diets is a rule rather than an exception. This is in accordance with previous findings in healthy children. Studies in germfree and conventional rats and mice have consistently shown high values of FTA in the germfree animals and most often zero values in samples from their conventional counterparts. It is also of interest that the transit times in germfree animals are usually somewhat prolonged compared to their conventional counterparts. Taken together, these findings indicate that inactivation of pancreas derived tryptic activity is mainly carried out by the intestinal microbial flora. Variations in composition of the flora may then lead to inter-species and inter-individual variations.

In the present study we have been working with the soluble part of the faecal tryptic activity. MacFarlane et al., investigating the distribution of proteolytic activity in different faecal fractions from five humans, found that 25–67 per cent of the
Table 2. Mean levels of faecal tryptic activity (FTA) prior to, during and after administration of antibiotics to healthy volunteers (doses, see materials and methods).

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of subjects</th>
<th>Prior to antibiotics</th>
<th>During antibiotics (day 6)</th>
<th>Following antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st Sample</td>
<td>2nd sample</td>
<td>After 1 week</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>6</td>
<td>70</td>
<td>110</td>
<td>30</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>7</td>
<td>120</td>
<td>160</td>
<td>130</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>6</td>
<td>40</td>
<td>70</td>
<td>40</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>7</td>
<td>130</td>
<td>80</td>
<td>60</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>6</td>
<td>160</td>
<td>140</td>
<td>220</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>6</td>
<td>50</td>
<td>150</td>
<td>140</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>6</td>
<td>30</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>7</td>
<td>60</td>
<td>80</td>
<td>70</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>7</td>
<td>80</td>
<td>160</td>
<td>110</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>7</td>
<td>150</td>
<td>80</td>
<td>70</td>
</tr>
</tbody>
</table>

Proteolytic activity was present in the particulate fraction. The most numerous proteolytic bacteria in the samples were identified as *Bacteroides* spp and *Propionibacterium* spp, known to have cellular proteases. Consequently, it was assumed that most of the proteolytic activity present in the sediment was of microbial origin, and that the soluble proteolytic activity could be of microbial as well as pancreatic origin. The high amount of soluble tryptic activity always found in faeces from germfree rats and mice indicates that pancreas derived trypsin activity is not attached to particles present in faeces. This is further supported by the findings that sonication of faeces from germfree rats does not increase FTA levels (unpublished data).

Table 2 shows that in connection with administration of ten different antimicrobial agents to groups of healthy volunteers, there were no significant alterations of the mean FTA levels. However, in some students, increased as well as decreased FTA levels occurred. The alterations might be caused, either by an influence upon the microbial inactivation of the pancreas derived trypsin or on the production of microflora derived proteases. The mere fact that microbes actually involved in the intestinal inactivation of trypsin are unknown, makes further speculations upon the mechanisms behind the observed drug effects of rather limited value.

The present data, as well as our previous data from germfree and conventional rats clearly show that the intestinal microflora has a key role in the regulation of intestinal breakdown of protein. We concur with MacFarlane et al., that further studies are required in order to identify proteolytic enzymes present in the colon, and to assess the qualitative effects of microbial and pancreas derived proteases on different protein available in this organ.

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