Effects of Tetracycline on Pancreatic Protein Synthesis and Secretion in Pigeons

P.C. Tucker, MD and P.D. Webster, MD

Studies presented herein examine the effects of tetracycline hydrochloride on pancreatic protein synthesis and secretion in pigeons, in vivo and in vitro. An unexpected finding was the impaired transport or secretion of amylase by the pancreas of treated animals. The studies indicate that tetracycline hydrochloride interferes with processes of secretion as well as synthesis and suggest that gastrointestinal dysfunction observed in patients receiving the antibiotic may, in part, be due to defective pancreatic protein synthesis and secretion.

Tetracycline and related compounds have been used extensively as broad spectrum antibiotics in the treatment of bacterial infections. It is well recognized that patients receiving such compounds in large doses or for extended periods of time may develop symptoms related to the gastrointestinal tract. Diarrhea is one of the more commonly recognized side effects. Reports have documented adverse effects of tetracycline on hepatic and small bowel function (1-4). Earlier reports demonstrated that tetracycline hydrochloride interfered with protein synthesis in both mammalian and bacterial cells (5, 6).

There are no reports indicating effects of tetracycline therapy on pancreatic function. The studies presented here measure effects of tetracycline hydrochloride administered in vitro and in vivo on pancreatic protein synthesis and secretion. These studies clearly demonstrate that tetracycline hydrochloride, as was anticipated, decreases pancreatic protein synthesis as measured by incorporation of L-phenylalanine-14C into proteins. However, an unexpected finding was the inhibition of transport or secretion of pancreatic proteins, specifically amylase, as determined both in vitro and in vivo. The studies suggest that diarrhea observed in patients on prolonged tetracycline therapy may result, in part, from impaired pancreatic digestive enzyme synthesis and secretion.

MATERIALS AND METHODS

Materials

White Carneau pigeons* (age, 6 to 8 weeks; weight, 450 to 500 g) were fed ad lib a mixture of cracked corn, millet, and wheat. All animals had free access to water. Composition and preparation of tissue culture media have been described (7).

Methods

In vitro studies. Tetracycline hydrochloride† was added to incubation flasks in amounts necessary to give the following concentrations: 1.0, 0.5, 0.25, 0.1 and 0.05 mg/ml. Tetracycline solutions were maintained at pH 7.4. In vitro incubation was accomplished in a shaking water bath for 60 minutes at 37°C. In vitro protein synthesis was estimated.

* Obtained from Palmetto Pigeon Farm, Sumter, South Carolina.
† E. R. Squibb & Sons and Nutritional Biochemical Corporation.
Table 1. In Vitro Effects of Tetracycline Hydrochloride on Pancreatic Protein Synthesis

<table>
<thead>
<tr>
<th>Concentration of tetracycline HC1 (mg/ml)</th>
<th>Incorporation of L-phenylalanine-14C (cpm/mg protein)</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1546 ± 314</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>1570 ± 306</td>
<td>0</td>
</tr>
<tr>
<td>0.10</td>
<td>1316 ± 174</td>
<td>-13</td>
</tr>
<tr>
<td>0.25</td>
<td>1299 ± 304</td>
<td>-16</td>
</tr>
<tr>
<td>0.50</td>
<td>1036 ± 265</td>
<td>-33</td>
</tr>
<tr>
<td>1.0</td>
<td>495 ± 85</td>
<td>-68</td>
</tr>
<tr>
<td>5.0</td>
<td>75 ± 7</td>
<td>-95</td>
</tr>
</tbody>
</table>

Values are means ± SE for 4 experiments.

*Estimated by measuring incorporation of L-phenylalanine-14C into protein; protein secretion was estimated by measuring amylase transport into supporting medium. Details of these methods have been described (8).

**In vivo studies.** Tetracycline hydrochloride was dissolved in physiologic saline and administered intramuscularly in doses of 50, 250, 125, and 6.25 mg/kg. When 50 mg/kg doses were administered, one-half of the material was injected into the right and one-half into the left pectoral muscles. Paired groups of control pigeons received saline intramuscularly. The animals were killed after 2, 4, 18, 48, and 72 hours.

The 50 mg/kg doses of tetracycline were associated with a mortality of 10%. No deaths were observed in animals receiving smaller doses of tetracycline.

In vivo effects of tetracycline hydrochloride on amylase secretion were studied in four groups of pigeons: a) one group given saline; b) one group given tetracycline hydrochloride (250 mg/kg) and killed 18 hours later; c) a third group given betahanechol chloride (2 or 4 mg/kg) and killed 30 minutes later; and d) a fourth group given tetracycline hydrochloride (250 mg/kg) and after 4 or 18 hours given 2 or 4 mg/kg betahanechol chloride and killed 30 minutes later. Amylase content in pancreas was then measured.

**Preparation and incubation of pancreatic slices.** Control and experimental animals were killed, pancreas removed, fat and excess connective tissue trimmed, and the organ placed in cold, freshly oxygenated Krebs-Ringer-phosphate buffer, pH 7.4. Pancreatic slices were prepared as previously described (7, 8); slices were incubated in flasks containing 500-mg slices, 5.0 ml tissue culture media (pH 7.4) and 10 μCi L-phenylalanine-14C. In vitro incubation was carried out in a shaking waterbath at 37°C for 60 minutes.

**l-phenylalanine-14C incorporation into protein.** At completion of in vitro incubation, the reaction was stopped by the addition of 5 ml 10% trichloroacetic acid containing cold phenylalanine; contents of the flask were homogenized using a ground glass homogenizer. Duplicate portions of homogenate were placed in 15-ml centrifuge tubes, additional trichloroacetic acid added and the material centrifuged. The precipitate was washed twice with 5 ml 5% trichloroacetic acid containing l-phenylalanine, once with 5 ml 95% ethanol and twice with 5 ml ether-ethanol mixture (3:1). After the last wash the supernatant was drained by inversion of the test tube. The precipitate was resuspended in 3 ml 0.3 N potassium hydroxide and the pellet dissolved by heating in a water bath at 90°C for 20 minutes. After solubilization of the washed precipitate, radioactivity and protein content were determined. These methods have been used previously (9).

**Measurement of amylase, protein and radioactivity.** For measurements of amylase content, 100 mg of pancreas from experimental or control animals was placed in 4 ml potassium phosphate buffer (pH 6.9); the tissue was homogenized and amylase activity determined by the method of Bernfeld using linter starch as substrate (10). An amylase unit represents amount of starch hydrolyzed to maltose in 3 minutes at 37°C. Protein content was determined by use of the biuret method as described by Gornall et al (11). Radioactivity was measured in a Packard Tri-Carb liquid scintillation counter using a scintillation mixture developed by Patterson and Green (12).

**RESULTS**

Table 1 shows in vitro effects of tetracycline...