Detection of staphylococcal enterotoxin in gastric juice

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SYNOPSIS The gastric juice of a patient showing symptoms of staphylococcal food poisoning was examined by a radioimmunoassay for the presence of enterotoxins. Assays gave markedly higher results at 35° than at 5°. The source for this discrepancy was attributed to interference due to trypsin activity on the basis of (1) the demonstration of hydrolysis of *p*-toluenesulfonyl-L-arginine methyl ester by the specimen, (2) inhibition of this activity by trypsin inhibitor from lima bean, and (3) lowered values produced for enterotoxins in gastric juice when the inhibitor was included in the assay system.

In episodes of staphylococcal food poisoning, where the suspected food is not available for analysis, tests for the presence of staphylococcal enterotoxin in gastric juice of patients may serve as a useful alternative. Rapid radioimmunoassays have been described for detection of staphylococcal enterotoxin types A, B, and C (Johnson *et al.*, 1971; Collins *et al.*, 1972; Collins *et al.*, 1973; Dickie *et al.*, 1973). However, our previous efforts to measure staphylococcal enterotoxin in stomach aspirate by radioimmunoassay (RIA) were unsuccessful since positive results were not corroborated by the microslide immunodiffusion test (Casman *et al.*, 1969). These attempts were expanded in the present study.

Material and methods

STAPHYLOCOCCAL ENTEROTOXINS

Purified enterotoxin A and rabbit antiserum were prepared in this laboratory (Robern *et al.*, 1975). Enterotoxin B and rabbit antiserum were from Makor Chemicals Ltd (Jerusalem). According to the manufacturer, the toxin was homogeneous in the analytical ultracentrifuge and by electrophoresis. Purified enterotoxin C₁ (Borja and Bergdoll, 1967) and rabbit antiserum were kindly made available to us by Professor M. S. Bergdoll, Food Research Institute, the University of Wisconsin (Madison).

RADIOIODINATION

Enterotoxin was trace-labelled with ¹²⁵I to a specific

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activity in the range 0.5 to 1.0 g-atom per mole, according to the method of Thorell and Johansson (1971). Iodide was separated from iodinated protein by filtration through a column of Sephadex G-100 which was equilibrated with saline-buffer (see below).

RADIOIMMUNOASSAY

Enterotoxin was assayed following the principles of Catt and Tregear (1967), as modified by Ceska *et al.* (1970). The procedure, adapted for this study from previous work (Dickie *et al.*, 1973), was as follows: antibody-coated tubes were prepared by incubating polystyrene test tubes (Falcon) overnight at 35° with 2.0 ml of a 1:10 000 dilution of antiserum in 0.1 M carbonate-bicarbonate buffer (pH 9.6), after which the tubes were washed with 0.9% (w/v) sodium chloride. Standard toxin solutions were prepared by diluting a portion of the stock standard to concentrations of 0.63-10 ng/ml with 0.05 M phosphate buffer (pH 7.4), 0.15 M sodium chloride, 0.05% (w/v) sodium azide, 0.05% (w/v) Tween 20, and 0.5% (w/v) bovine serum albumin (Sigma lot no. 43C2300). The gastric aspirate was diluted with the same solvent giving dilutions of 36, 72, 144, and 288 fold. The usual assay mixture consisted of ¹²⁵I-enterotoxin (about 50 000 counts/min) and 1.0 ml of standard toxin or diluted aspirate in a final volume of 1.5 ml. After incubation at 35° or 5° for 16 hours the tubes were washed twice with tap water and count rates were measured in a gamma counter. The correction for non-specific bound counts/min (less than 0.5%

of the total radioactivity) was determined after incubation in uncoated tubes in the absence of unlabelled toxin. The ratio of bound to total radioactivity for ligand at zero dose was in the range of 0.15 to 0.20. The dose-response curve for standard toxin was

Fig 1 Hydrolysis of p-toluenesulfonyl-L-arginine methyl ester by stomach aspirate: 70 μ l (●), 30 μ l (○), 30 μ l plus 1 mg lima bean trypsin inhibitor (×).

Fig 2 Inhibition of binding of 125 I-enterotoxin A to rabbit antiserum by enterotoxin A and stomach aspirate: enterotoxin A (●), stomach aspirate (○), stomach aspirate plus lima bean trypsin inhibitor added at 1 mg/ml of 1:36 dilution (×). Each point is the average of two determinations (SE for average bound counts/min $\approx$ 5%) using 1.00 ml of serial dilution of standard toxin or stomach aspirate. The temperature of incubation was 5°. The presence of lima bean inhibitor in enterotoxin solutions (0.5 mg:10 ng toxin) did not alter the characteristics of the standard curve.

linearized using the relationship $\logit (B/B_0) = a + b \log(x)$ (Rodbard *et al*, 1969). Values for 'a' and 'b' were obtained by unweighted least squares regression (Magar, 1972).

TRYPSIN ACTIVITY

This was assayed using p-toluenesulfonyl-L-arginine methyl ester according to the procedure in 'Worthington Enzymes Reagents', Worthington Biochemical Corp, Freehold, New Jersey.

Results and discussion

A stomach aspirate was obtained from a 21-year-old woman showing symptoms typical of staphylococcal enterotoxigenesis. Approximately 5×10^5 viable *Staphylococcus aureus* organisms were found per millilitre. Five *Staph. aureus* colonies were sub-cultured for further tests. Tests for enterotoxin types A, B, C, D, and E by the microslide method showed that only one isolate of *Staph. aureus* produced enterotoxin (type A) in culture. RIA carried out at 35° gave strongly positive results (about 10 μ g/ml aspirate) for each of the enterotoxins (types A, B, and C₁) tested but assays at 5° led to five-fold lower values. Analysis of the aspirate by the microslide method, which in our hands can detect 0.5 toxin/ml, gave a negative result for each toxin (A, B, C₁).

A possible reason for these results was suggested by the presence of trypsin activity in the specimen (fig 1). In the following experiments, trypsin inhibitor from lima bean (Worthington) was added to the assay tubes at zero time at the previously determined

ratio (1 mg:30 μ l aspirate) to yield complete inhibition of the trypsin activity in 4 minutes (fig 1). A shift in the dose-response curve for enterotoxin A (fig 2) and B (not shown) to the low-dose region of the standard curve was seen, giving values of 0.07 and 0.05 μ g/ml, respectively. For the same dilution range, the dose-response curve for enterotoxin C₁ in assays using inhibitor was shifted beyond the detection limit of the assay, which usually occurs at about 90% B/B₀ (Rodbard *et al*, 1969). A similar effect was seen in assays at 5° comparing untreated and heat-treated (for 10 minutes at 80°) specimens, but values were further reduced (eg, 0.03 μ g enterotoxin A/ml and no detectable type B or C₁). These findings can be explained by assuming the digestion of antibody and/or antibody-¹²⁵I-toxin complexes by trypsin activity, giving erroneously low values for ¹²⁵I-activity bound to antibody. On the other hand, positive results for enterotoxin in the presence of trypsin inhibitor, or after heat-treatment, were interpreted as reflecting the presence of enterotoxin-producing *Staph. aureus* in the specimen. With the latter treatment, some loss due to thermal inactivation of toxin is expected. It seems that RIA might have some merits in the analysis of gastric juice for enterotoxin. However, it is evident that interference by proteinase should be considered when such specimens are examined. A problem encountered in the detection of hepatitis B virus in human faeces and bile has also pointed to the involvement of proteolytic enzymes (Moodie *et al*, 1974).

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