LETTERS TO THE EDITOR

Nitrict oxide synthase in gastric mucosa

EDITOR—Rachmilewitz et al have reported (Gut 1994; 35: 1394–7) increased nitric oxide synthase activity in the antral and fundal gastric mucosa from patients with duodenal ulceration and Helicobacter pylori infection. Nitric oxide synthase activity was estimated from citrulline production.

However, we have measured citrulline concentrations in gastric antral biopsy specimens (by LKB biochrom amino acid analyser) as an index of this enzyme’s activity and found no differences before and after healing duodenal ulcers by eradication of H pylori.

In addition we estimated nitric oxide synthase activity in the cytosolic supernatants of antral mucosal biopsy specimens from the inhibition by monomethyl-L-arginine (L-NMMA) on the conversion of 14C L-arginine to 14C citrulline. Initially we used the same method as used by Rachmilewitz et al, and estimated 14C citrulline concentrations using Dowex AG 50W-X8 (sodium form) columns. However, to validate this method we applied the fraction recovered from the column to thin layer chromatography. 14C urea, 14C ornithine, and other unidentified radiolabelled products were found in addition to 14C citrulline. Therefore, we conducted the experiments using thin layer chromatography to avoid possible inaccuracies of the Dowex column method. Once again we did not find an increase in nitric oxide synthase activity in H pylori positive duodenal ulcer patients, but discovered that in this tissue L-NMMA significantly inhibited the formation of 14C citrulline.

Demonstration of nitric oxide synthase activity in the Rachmilewitz study depends upon the inhibitory effect of monomethyl-L-arginine (incorrectly labelled L-NAME in their paper) on 14C citrulline, which was estimated from the scintillant activity of the Dowex elute thought to arise from citrulline alone. As we have shown that this elute contains other radiolabelled metabolites of arginine, it is possible Rachmilewitz et al are measuring the inhibitory effect of L-NMMA on the formation of other labelled substances such as 14C ornithine from 14C arginine by glycine aminotransferase.

The Dowex AG 50W-X8 separation as a measurement of nitric oxide synthase activity originally described by Bredt and Snyder2 was validated by thin layer chromatography for cerebellar tissue. It may not be accurate when applied to mucosal samples and, as suggested by them, should be validated by thin layer chromatography for each new tissue.

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Mycobacterium paratuberculosis and Crohn’s disease

EDITOR—We have studied the participation of mycobacteria in Crohn’s disease and are interested in articles published on this topic.

The paper of H M Fidler et al (Gut 1994; 35: 506–10) clearly highlighted the ubiquitous nature of Mycobacterium paratuberculosis and its detection with the highly sensitive and specific tool of polymerase chain reaction in human tissue.

In our study (unpublished data) we were specifically interested in the group of patients with Crohn’s disease with terminal ileum involvement, although three patients with classic Crohn’s colitis alone on histological examination, were also included.

Polymerase chain reaction assays based on the direct amplification of 229 bp fragment and 400 bp of the repetitive mycobacterial insertion sequence IS900 present in the genome of M paratuberculosis, were applied to 73 sections of paraffin wax embedded tissue of 26 histologically confirmed Crohn’s disease patients, all of which were negative on Zielh-Neelsen staining for acid fast bacilli.

These sections were divided into those with an area of minimal involvement and those with an area of well established ulceration.

In 18 cases lymph nodes were also available for study. Granulomata were present in only one of 23 of 73 sections.

As the incidence of pulmonary tuberculosis in South Africa is among the highest in the world, the presence of M tuberculosis was also investigated, to exclude this organism as a complicating factor.

Controls consisted of patients with colon cancer who had undergone a right hemicolectomy (n=54, 102 samples). No positive amplification of the 400 bp sequence in any of the 26 patients after 40 cycles was found. In some of the 229 bp sequence amplification reactions, a slight product was found, pointing to the presence of M paratuberculosis. Reamplification of the 229 bp products was done and positive reamplification was detected in 10 of 26 (38%) of Crohn’s disease patients and in four of 35 (11%) controls.

There was a greater chance of detecting the organism in non-granulomatosus sections (17% of 12 41 (29%) than in sections showing granulomata of one of 23 (4%) patients.

There were no cases of Crohn’s disease that were associated with tuberculosis. At eight terminal ileum samples with Johnie’s disease confirmed by culture and histological examination tested positive for M paratuberculosis DNA. The internal laboratory controls for the reamplification of the reamplification were repeatedly tested negative.

These initial results suggest that M paratuberculosis does not play a part in the pathogenesis of Crohn’s disease. The detection of M paratuberculosis DNA in 38% of Crohn’s disease patients on reamplification, however, seems to contradict this finding.

We find it interesting that in the study of Fidler et al none of the small bowel tissues were positive for IS 900 for M paratuberculosis with all the positive reactions being found in the large bowel specimens.

As two of three of our patients who had confirmed Crohn’s coliit in patients with IS 900 for M paratuberculosis, the question arises as to whether this organism is more likely to be associated with Crohn’s disease activity of the large bowel rather than the terminal ileum.

Failure of bowel culture to detect M paratuberculosis in Crohn’s disease after 40 cycles combined with a low detection rate even after reamplification suggests that there is a possibility association between M paratuberculosis and the pathogenesis of Crohn’s disease remains uncertain.

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