Immunoreactive Inhibin-like Material in Duodenal Ulcer Patients and Controls

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Abstract

Immunoreactive inhibin-like material (IR-ILM) was measured by radioimmunoassay (RIA) in serum and fasting gastric juice samples of 34 normal men and 70 men with duodenal ulcer (50 for serum analysis). The mean concentration of IR-ILM in the gastric juice (59.7 ± 7.5 ng/mg protein) and serum (29.8 ± 2.8 ng/ml) of duodenal ulcer patients was significantly higher (P < 0.01) than in the gastric juice (23.1 ± 4.6 ng/mg protein) and serum (17.4 ± 2.3 ng/ml) of normal men. Demonstration of inhibin-like peptide in gastric juice is an original contribution in the emerging list of gut hormones.

Key words: Inhibin, duodenal ulcer, gastric juice, serum

Introduction

In the past decade our understanding of gut hormones has increased remarkably owing to discoveries of new peptides and their basic characteristics.

In this study we report the detection of a high concentration of another peptide, inhibin, in the gastric juice of normal men and patients with duodenal ulcer (DU). Earlier we had reported levels of immunoreactive inhibin-like material (IR-ILM) in 22 patients with DU, whereas the present investigation is on a larger sample size of 70 patients and the concentration of IR-ILM is studied with respect to total protein in gastric juice. Inhibin is a polypeptide hormone secreted by the antral follicles in females and the Sertoli cells in males. It has been detected and partially purified from follicular fluid and seminal plasma of several species including man. Besides the gonads, inhibin has now been shown to originate from the prostate and placenta and has multiple mechanisms of action. It decreases the binding of follicle stimulating hormone (FSH) to testis and brings about reduction in FSH induced cAMP accumulation in the testis. Inhibin also interacts with thyrotropin releasing hormone (TRH), thereby modulating prolactin release.

Material and Methods

Serum and gastric juice samples from 34 fasting normal healthy men, gastric juice from 70 men with DU and serum samples from 30 of these DU patients were collected at the Lokmanya Tilak Municipal Hospital, Bombay. The age of normal men and DU patients varied from 30-50 years. All subjects studied were admitted to the hospital and kept fasting overnight from 22.00 h to 10.00 h and thereafter subjected to fiberoptic endoscopy. This was immediately followed by collection of gastric juice and blood samples. Samples of gastric juice and blood were in cold throughout the experimental procedure. After collection each sample was centrifuged at 800 g at 4°C for 10 min. The pH of the supernatant was recorded using pH meter and adjusted to 7.0 using 1 M NaOH. The supernatant and serum samples were stored at −20°C until analysed for inhibin-like material (ILM). The concentration of ILM in gastric juice was expressed per mg of protein. The protein content of the gastric juice was determined by the method of Lowry et al. A homologous preparation of inhibin isolated from human seminal plasma was used as a reference standard and as antigen for radioiodination by Iod. Antibodies to inhibin were raised in rabbit by active immunization. In vivo and in vitro neutralization experiments were carried out to ensure that the antibodies formed were against the biologically active site of inhibin. This antiserum was capable of binding 50% of radioiodinated inhibin at a dilution of 1:10,000.

Radioimmunoassay

A homologous preparation of inhibin isolated from human seminal plasma was used as a reference standard and as antigen for radioiodination by Iod. Antibodies to inhibin were raised in rabbit by active immunization. In vivo and in vitro neutralization experiments were carried out to ensure that the antibodies formed were against the biologically active site of inhibin. This antiserum was capable of binding 50% of radioiodinated inhibin at a dilution of 1:10,000.

Results

Fig 1 shows the concentration of IR-ILM in the gastric juice of normal men and patients with DU. The mean IR-ILM concentration (59.7 ± 7.5 ng/mg protein) in DU patients was significantly higher (t = 2.94, P < 0.01)
Fig 1: IR-ILM levels expressed per mg protein in gastric juice of normal men and patients with duodenal ulcer.

than in normal men (23.1 ± 4.6 ng/mg protein). Twenty percent (14/70) of DU patients showed IR-ILM levels above the uppermost level of 90 ng/mg protein found in the gastric juice of normal men. There was no statistical difference in protein concentration in gastric juice of normal men (2.2 ± 0.2 mg/ml) and DU patients (2.1 ± 0.15 mg/ml) (t = 0.390).

As shown in Fig 2 DU patients had significantly higher (t = 3.26, P < 0.01) mean serum IR-ILM concentration (29.8 ± 3.3 ng/ml) than in normal men (17.4 ± 2.3 ng/ml). Fifteen of 50 DU patients had serum IR-ILM concentration above the uppermost level of 43 ng/ml found in normal men. No significant correlation was observed between serum and gastric juice IR-ILM levels in control subjects as well as in DU patients, the correlation coefficient being 0.29 and 0.18 respectively.

Discussion

Inhibit preparations isolated from different sources are reported to differ in their molecular weights; however, all of them share the common property of selective reduction of FSH secretion. Due to the controversy regarding the molecular size of inhibit, a peptide which selectively suppresses FSH secretion is designated inhibit-like material. It may be added here that the complete amino acid sequencing of inhibit preparation from human seminal plasma was first reported from this laboratory.15-17 We have partially purified inhibit-like material from gastric juice. However, amino acid sequencing of this material has not been carried out. Hence we have referred to this material, detected by radioimmunoassay, as immunoreactive inhibit-like material. However, it may be noted that when gastric juice proteins were co-chromatographed with radiolabelled human seminal plasma inhibit most of the immunoreactive inhibit from gastric juice was recovered in the peak corresponding to the peak recovery of radiolabelled inhibit,15 and also inhibit-like material in gastric juice shows FSH suppressing bioactivity as defined for inhibit.18

We have demonstrated the presence of IR-ILM in gastric juice and serum of patients with DU as well in normal men. DU patients had higher mean IR-ILM concentrations in both gastric juice and serum as compared to the control group. Fourteen of 70 gastric juice samples and 15/50 serum samples from DU patients showed higher IR-ILM levels than the uppermost limit found in normal men.

The exact site of synthesis and secretion of inhibit in the stomach is not yet known. Its role as a marker in carcinoma of the stomach has been suggested.19

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BOOK REVIEW

A Hard Look at the Pancreas


Professor P J Geavrich has played a stellar role in bringing tropical calcific pancreatitis (TCP) to the notice of the medical fraternity. He deserves praise for a well studied and lucidly written book on a subject that is baffling the medical profession.

TCP is seen in southern India and more specifically in Kerala, and occurs in the younger age group. TCP is quite distinct from alcoholic calcific pancreatitis (ACP) in the middle aged population of western countries. Calcific pancreatitis exhibits either stones in the pancreatic ducts or calcified pancreatic parenchyma. Acute pancreatitis does not precede TCP. TCP is characterized by episodic abdominal pain in childhood, diabetes and calcification of the pancreas. TCP is associated with a higher prevalence of diabetes (90%) and low prevalence of steatorrhea (4-10%), and these patients are more likely to die of hypoglycemia, cirrhosis of the liver, and acute or chronic infection, renal failure or pancreatic cirrhosis. On the other hand ACP has a lower prevalence of diabetes (0-5%) and higher prevalence of steatorrhea (95%), and death is due to cardiovascular disease, surgery or non-pancreatic malignancy.

Pancreatic biopsy from operated patients of TCP reveals dilatation of the ducts and ductules containing amorphous material, loss of duct epithelium, calcospherites and inflammatory exudate, mainly lymphocytes and plasma cells. The acinar tissue is replaced by fibrous tissue while islet tissue is often preserved and is sometimes initially hyperplasia but later atrophied. These changes could be experimentally produced in monkeys by ligating the pancreatic duct, indicating that ductal obstruction is primarily responsible for the changes in TCP.

The mechanism of ACP is presumed to be precipitation of protein of pancreatic enzymes in the pancreatic ductules. TCP occurs in those with single diet of cassava that has low protein (0.7%) and high carbohydrate. However lower protein intake in kwashiorkor does not produce pancreatic calcification. Cassava contains hydrocyanic acid. Sulphur-containing amino acids, methionine and cystine are necessary to delay the effect of cyanide but these amino acids are deficient in cassava. The good news is there is a decreasing consumption of cassava in Kerala State and in recent years there is a corresponding decrease in patients admitted to hospitals with calcific pancreatitis.

The printing is of very high standard and most figures are of top quality. This book is indispensable to all gastroenterologists and medical libraries.

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