Morphological, biochemical and autoradiographic studies of pancreatic acinar cell necrosis and its regeneration induced by 4-hydroxyaminoquinoline-1-oxide in rats

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Summary: A single intravenous injection of 4-hydroxyaminoquinoline-1-oxide (4-HAQO) induced a dose-dependent diffuse pancreatic acinar cell necrosis within 48 hours after the administration in rats. Regeneration following the necrosis ensued by 72 hours. The serum amylase levels were markedly elevated within 24 hours, which paralleled a decrease of digestive enzyme contents in the pancreas. The change of these biochemical parameters corresponded well with the morphological change of the pancreas. Autoradiographic studies with the 3H-thymidine revealed that the labeling index of the regenerating acinar cells was considerably higher, reaching a peak value of nearly 18.2% at 72 hours after injection of 4-HAQO and it still remained high in 2.67% even at 168 hours after injection. Gastroenterol Jpn 1989;24:181–194

Key words: Autoradiography, Experimental acute pancreatitis, Pancreatic enzymes, Regeneration of Pancreatic tissue

Introduction

Acute pancreatitis is assumed to be caused by many kinds of triggering factors and autodigestion by pancreatic enzymes has been considered to play an important role in the pathogenesis of acute pancreatitis. In regard to pancreatic regeneration, the capability of regeneration in the adult pancreas has generally been minimal, if at all. On the other hand, positive evidence supporting pancreatic regeneration from experimental studies has been documented in the past three decades. Many different kinds of experimental models induced acute pancreatitis have been hitherto documented. Konishi et al. reported pancreatic acinar cell damage induced by 4-hydroxyaminoquinoline-1-oxide (4-HAQO) in rats in 1974. 4-HAQO was used in this study. This compound should be given special attention because it can induce not only acute pancreatic acinar cell damage but also pancreatic cancer. We reviewed different patterns of pancreatic damage, depending upon the route and dose of 4-HAQO administered, and described that the acinar, as well as...
lobular, architecture was restored by the fourth week after administration of 4-HAQO. In a previous paper, we gave a detailed electron microscopic account of subcellular events of regeneration following acinar cell necrosis induced by 4-HAQO. This article presents the exact morphological, biochemical and autoradiographic details of sequential events in the process of regeneration following acinar cell necrosis induced by 4-HAQO up to 168 hours, which have not been reported before.

Materials and Methods

General procedures

Male rats of Wistar strain (Shizuoka Animal Co., Shizuoka, Japan) weighing about 170 grams were used. The animals were housed under normal condition (room temperature: 20-25°C), and were fed laboratory chow (CE-2, Nihon Clea Co., Tokyo, Japan) and water ad libitum, upon the time of sacrifice. In the experimental groups, acute pancreatic acinar cell necrosis was induced by injection of 4-HAQO at different doses of 7, 14 or 21 mg/kg, body weight into the saphenous vein under ether anesthesia. A corresponding volume of 0.005 N HCl was given to the control rats. For intravenous injection, 4-HAQO was dissolved in 0.005 N HCl solution immediately before the experiment. Five rats in each group were sacrificed at 24, 48, 72 and 168 hours after injection of the agents. Under ether anesthesia, blood was collected from the abdominal aorta. The blood was allowed to clot at room temperature and serum was collected after low speed centrifugation in a refrigerated centrifuge. Pancreata were quickly removed and weighed with an electronic reading balance (Shimazu, Libror, EB-280) after careful separation of the surrounding connective tissue. After weighing the pancreas wet weight, 10% homogenates in ice-cold normal saline were prepared. Homogenization of pancreata was performed with a Polytron (Model PT 10, Brinkman Instrument, Webury, NY) set at scale 10 for one minute in 10% homogenate with normal saline.

Morphology

A part of pancreatic tissue was fixed in 10% neutral buffered formalin and embedded in a paraffin section. Sections, 5μm were routinely stained with hematoxylin and eosine (H and E). In the rats given 14 mg 4-HAQO/kg, body weight, Masson’s trichrome staining was performed to confirm proliferation of interstitial connective tissues at 168 hours after injection.

Amylase assay

Amylase was measured on the same day when sacrifice was done. Serum amylase was determined on 0.1 ml of aliquots sera diluted 20-fold with 20 mM-PO₄ buffer, pH 6.9, containing 6 m mol of NaCl. Amylase in the pancreas was determined on 0.1 ml of sample diluted 400-fold with the same phosphate buffer.

Measurement of serum isoamylase

Serum isoamylase was measured on aliquots stored for up to 4 days at -40°C, and then thawed before use. Electrophoretic analysis of serum amylase was based on TITAN-III-LIPO (Cellulose acetate plates, Helena) electrophoresis using the discontinuous buffer system of Kohn at 250 V for 40 minutes. Enzyme activity was visualized by the method of Davies. Isoamylase fractions were measured using densitometry (Corning Model 760) at 620 nm.

Assay of trypsinogen and chymotrypsinogen

Trypsinogen and chymotrypsinogen were measured on aliquots of homogenates stored for up to 4 days at -40°C. One ml of 10 mM Tris buffer, pH 8.5, containing 0.1% Triton X-100 and 0.9% NaCl was added to an equal volume of 10% homogenate in normal saline. These samples were sonicated for one minute, set at scale 4 using a sonicator cell disruptor (Model W-10, Heat System-Ultrasonic Inc, Plainview, NY) and then diluted 20-fold with 80 mM Tris buffer, pH 7.8, containing 100 m mol CaCl₂. One ml of diluted homogenate was incubated at 37°C for 120 minutes with an equal volume of 0.05% enterokinase in the same buffer used for dilution of the homogenates. Under these condi-