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Acute experimental pancreatitis in rat induced by sodium taurocholic acid: objective quantification of pancreatic necrosis

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Summary. Retrograde injection of 5% sodium taurocholic acid (TA) in Wistar rat pancreatic duct is followed by acute pancreatitis, resulting in 100% mortality within 36 h. Biochemical determinations show raised levels of amylase in ascites and blood. Necrosis has been measured using seven morphometric characteristics of pathological changes that add precise information on the type and extension of the pancreatic lesion. The percentage of necrotic tissue (by area) seems to be the most objective parameter. Necrosis appears 6 h after TA infusion, being 5.77% in extent after 12 h, 14.9% after 24 h and animals die with an area of 29.5% necrosis. This experimental model seems to one in which physiopathological and therapeutic trials on acute pancreatitis may be camed out.

Key words: Acute experimental pancreatitis – Sodium taurocholic acid – Pancreatic necrosis

Introduction

Human necrohaemorrhagic acute pancreatitis in its different clinical forms (multiorgan failure), with distinct biochemical (hyperamylasaemia) and pathological (pancreatic and peripancreatic necrosis) features has been studied in different experimental models. Retrograde injection of sodium taurocolic acid (TA) into the rat pancreatic duct is followed by progressive pancreatic necrosis, proportional to the concentration of the stimulating agent (Aho et al. 1983a). Other experimental models can be classified into non-lethal (cerulein, Watanabe et al. 1984), intermediary (ethionine-supplemented choline-deficient diet, Lombardi et al. 1975) and duct ligature (Zelander et al. 1964) and lethal forms (pancreatic ischaemia, Popper et al. 1948) and retrograde injection of stimulating agents like enterokinase (Terry et al. 1987) or taurocholic acid (Aho et al. 1980a).

The aim of this paper is to add objective and quantifiable pathological information to the model of necrohaemorrhagic pancreatitis by ductal retrograde injection of TA in the Wistar rat. This assists us to evaluate the physiopathological and therapeutic value of this model.

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Materials and methods

Sixty-five male Wistar rats (weight ranging from 380 to 420 g) were randomized into three groups:

Group 0: ($n=5$). Anaesthesia was achieved by intraperitoneal administration of 1 ml/100 g of a combination of ketamine (5 ml, 10 mg), diazepam (2 ml, 10 mg) and atropine (2 ml, 2 mg). After rats had been anaesthetized an abdominal midline incision was made and blood from caval and portal veins obtained by direct puncture. Pancreatoduodenal and spleen en-bloc resection was performed. Both biochemical and pathological values obtained, were considered to be normal for further considerations.

Group 1: ($n=30$). Anaesthesia was similar to group 0. After the incision had been made, the proximal duodenum was exposed and punctured with a fine cannula which was placed in the common biliopancreatic duct (Aho et al. 1980a). A soft surgical clamp was placed in the biliary duct, just distal to the liver. In the same way, another clamp was placed around the bile duct at its point of entry into the duodenum to avoid reflux of duodenal contents into the pancreatic duct. Continuous infusion of isotonic saline (0.9%) at a constant flow of 0.1 ml/min was then started; it was maintained for as many minutes as there were hundreds of grams weight in the animal used, resulting in a variable volume infused. Flow was controlled by an exact perfusion pump (Braun Perfusor Secura München, FRG). Once the perfusion was finished and surgical clamps put aside, the abdomen was closed and the animals allowed to recover. Blood and pathological samples were obtained in the same way as in group 0 at 12 h (group 1A; $n=10$), 24 h (group 1B; $n=10$) and 36 h (group 1C; $n=10$).

Group 2: ($n=30$): The only difference from group 1 was that instead of isotonic saline, 5% TA (T-4009, Sigma, St. Louis, MO, USA) was infused at the same quantity. Samples were taken at 12 h (group 2A; $n=10$), 24 h (group 2B; $n=10$) and 36 h (group 2C; $n=10$).

Biochemical analysis included amylase among other parameters. Pancreatic tissue was fixed in 10% formaldehyde, embedded in paraffin, cut and stained with haematoxylin and eosin. Morpho-

metric studies were made with a semi-automatic image system (CUE-2, Galai software version 2.2, 1987, MT002236A Jerusalem, Israel). Seven histological parameters of necrosis were studied: percentage necrotic area (PNA), density expressed as number of foci per square centimetre (DSC), median necrosis area (MNA), the perimeter of foci of necrosis (PNF), maximum (MaNF), mean (MeNF) and minimum (MiNF) Feret's diameters of the necrotic foci. All measurements, except for the PNA, were made in micrometres.

Feret's diameter is the measure of the projection of the diameter of the necrotic foci at specified angles (0°, 45°, 90° and 135°), MaNF being the length of the Feret's longest diameter and MiNF the length of the shortest. MeNF is obtained as the mean of the diameters measured at 0°, 45°, 90° and 135°. PNA seems to be the more objective variable because it does not depend on the size of the pancreas.

Statistical analysis of results was made with a non-parametric test (Mann-Whitney) with two-tailed probability test when saline and TA groups were compared. The Kruskall-Wallis test with two confidence intervals was used to compare group 0 versus group 1 versus group 2. Survival obtained in each group was compared with Breslow and Mantel-Cox tests.

Results

Results obtained for amylase in both group 1 and 2 are expressed in a bar chart (Fig. 1). The group with intraductal infusion of saline (Group 1) remains within the physiological levels of amylase; progressive elevation of amylase levels can be noticed in the group in which TA was infused (group 2), as a biochemical index of acute pancreatitis (Table 1).

After 12 h, amylase levels reached statistical significance ($P<0.001$) between group 1A (saline) and group-2A (TA) and also when compared with the control group (group 0) ($P<0.001$). A statistical difference was also noticed between group 1B and 2B (24 h).

Mortality was only seen in those rats in which TA was infused, being 100% after 36 h. No mortality was found in group 1 ($P<0.003$) (Table 1).

In group 1 (saline) minor histopathological damage (oedema) was noticed in the first hours of evolution (group 1A), but no necrosis was recorded. In the group of rats in which TA was infused (group 2), pancreatic necrosis was progressive and quantified. It appeared as dispersed foci of coagulative necrosis with slight, or without any acute inflammatory infiltrate. Necrotic foci were localized in centrilobular areas, and when confluent, only a rim of viable peripheral acini was seen. In some animals there was fat necrosis with saponification.

All seven variables were measured and are given in Table 2 and Fig. 2 (PNA). Mean and standard error (SE) are shown in Table 2.

As no necrosis was found in the saline group a marked statistically significant difference ($P<0.001$) was

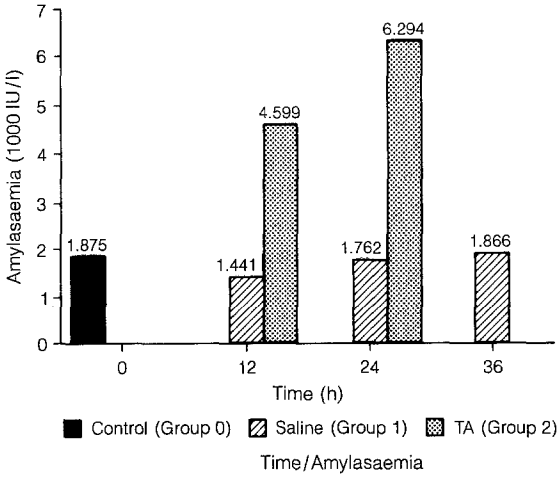

Fig. 1. Amylase in group 1 (saline) remains within physiological levels while progressive elevation can be noticed in group 2 (taurocholic acid)

Table 1. Amylase (mean ± SE) and mortality

Group	Amylase (1000 Iu/l)	Mortality (%)
0	1.87 ± 1.42	0
1A	1.44 ± 1.41	0
1B	1.76 ± 2.52	0
1C	1.86 ± 2.03	0
2A	4.59 ± 8.42	0
2B	6.29 ± 2.20	0
2C	–	100%

Table 2. Results of measurements

	Group			Significance
	2A	2B	2C	
PNA (%)	5.77 ± 2.36	14.90 ± 4.23	29.52 ± 17.08	2A–2B, 2A–2C
DSC	17.88 ± 4.78	49.28 ± 28.24	65.92 ± 43.90	2A–2B, 2A–2C
MNA (× 1000)	321.52 ± 611.33	346.15 ± 91.86	628.70 ± 417.80	–
PNF	2010.61 ± 646.03	2204.83 ± 496.51	3615.91 ± 810.80	2A–2C, 2B–2C
MaNF	799.67 ± 338.18	937.64 ± 209.04	1231.79 ± 227.70	–
MeNF	626.61 ± 184.50	729.57 ± 169.51	946.41 ± 165.77	2A–2C
MiNF	429.38 ± 101.82	500.61 ± 145.42	674.41 ± 115.98	2A–2C

PNA, percentage area of necrosis; DSC, density per square centimetre; MNA, mean necrosis area; PNF, perimeter of foci of necrosis; MaNF, maximum Feret's diameter of foci of necrosis; MeNF, mean Feret's diameter of foci of necrosis; MiNF, minimum Feret's diameter of foci of necrosis

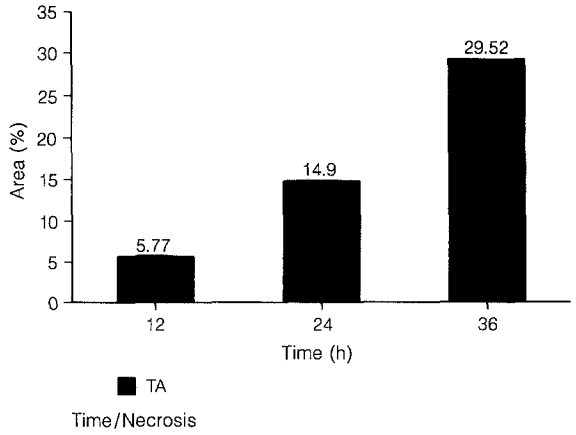

Fig. 2. Percentage area of necrosis. Necrosis appeared within 12 h and was 29.528% at death

found between groups 1 and 2 in respect to the characteristics of histopathological necrosis.

Discussion

Many cases of human acute pancreatitis do well with conventional therapy. However, some necrohaemorrhagic forms have a fatal outcome, and in these cases no treatment has been shown to be of value. Experimental and therapeutic trials focus on this type of pancreatitis in order to improve management of these patients (Seeling et al. 1975; Glazer and Bennet 1976; Seeling and Seeling 1976; Sanfey et al. 1986).

Among the different models of experimental pancreatitis, the one based on the Heinckel technique (Heinkel 1953) of retrograde intraductal infusion of TA fits well with these requirements, because it produces a necrohaemorrhagic pancreatitis, with death of all the animals within 36 h. This is especially true when flow and infusion pressure is controlled, one of the main factors in the consistency of experimental pancreatitis (Steer 1985). Pancreatic (Popper et al. 1948; Theve et al. 1973; Aho et al. 1980a, b) and extra-pancreatic (Aho et al. 1983b) lesions are well documented and are similar to those which appear in man (Simon and Giacobino 1970; Kitamura et al. 1973; Nevalainen 1980; Rao et al. 1980; Aho et al. 1983b). However, pathological features of pancreatic and peripancreatic necrosis have not been quantified using objective determinations.

In our study, no necrosis was found in group 1 (saline). Necrosis in the TA group cannot be attributed to surgical technique therefore, nor to flow or infusion pressure, but only to the irritant itself.

In the animals into which TA was infused (group 2), objective pancreatic necrosis appeared within 12 h, and progressed until 36 h, as we can deduce from the PNA, with statistically significant differences between groups 2A and 2B, and 2A and 2C (Table 2). This increase is mainly due to a greater number of necrotic foci and not to an increase in their size, as the DSC, MNA, MaNF, MeNF and MiNF show (Table 2). Nevertheless, foci tend to be greater in volume (MNA) in pancreatitis at 36 h (Table 2), although no statistical significance is reached between any group, perhaps because of the irregularity (high standard deviation) of focus size. However, variables related to size and contour of foci (PNF, MeNF and MiNF) show increased values in evolving pancreatitis (Table 2).

These findings suggest that once injury is produced by the irritant necrosis appears, is progressive, and is later maintained without further injury. PNA at death was 30%, but in Aho's studies (1983a) some animals died with only 10% PNA. This is probably due to the extra-pancreatic changes (Carey 1979) observed in acute pancreatitis.

In spite of the lack of relationship between clinical and experimental acute pancreatitis in physiopathological terms, the pancreatic necrosis produced in the present model is very much like that observed in man.

This standardized model, in both its technical and biochemical aspects as well as in its histopathological and quantitative features, will allow therapeutic and evolutionary studies of acute necrohaemorrhagic pancreatitis based on objective criteria. These studies can be carried out not only in severe pancreatitis, but in milder forms produced by changes in the irritant concentrations (Aho et al. 1983a).

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