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Antecedent long term ethanol consumption in combination with different diets alters the severity of experimental acute pancreatitis in rats

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SUMMARY The effect of fat rich (F), protein rich (P) and carbohydrate rich (C) diets and chronic ethanol consumption on experimental acute pancreatitis was studied in rats. One hundred and ninety two animals with induced acute pancreatitis were divided into eight groups fed either a mixture of water and 15% ethanol (v/v), or tap water combined with standard or special diets according to their group, for 12 weeks. The other control 192 rats were divided into equal groups. Bile induced experimental acute pancreatitis caused the highest mortality (37·5%) in the animals receiving F diet and ethanol. In this group significant haemoconcentration, peritoneal exudate formation and the most extensive fat necrosis were also observed. The carbohydrate rich diet with or without ethanol did not have any significant effect on the severity of acute pancreatitis. Diet and ethanol may alter the metabolism of the pancreas and cause derangements at the systemic level. These derangements might cause the increased susceptibility to acute pancreatitis. These changes in the metabolism may be fatal because of the increased toxicity of the peritoneal exudate secreted during the inflammation.

Alcohol is a common predisposing factor in acute pancreatitis, but the role of diet and other environmental factors have remained obscure.[1] Durbec and Sarles have observed some clinical evidence to support the theory concerning the relationship between diet and acute pancreatitis.[2] Controlled studies of human dietary habits in connection with acute pancreatitis, however, are difficult to arrange.

To solve this problem two experimental studies have been presented in the literature. Haig[3] and Maki *et al*[4] both found a significant effect of special diets on the severity of the experimental acute pancreatitis. Their results were somewhat conflicting: a fat rich diet intensified experimental acute pancreatitis in dogs, but in Maki's work rats receiving the protein rich diet developed the most severe acute pancreatitis.

It has been suggested that alcohol alone may not make the pancreas more vulnerable to inflammation and that some additional factor is needed.[5] Diet could be such a factor, because fat rich diets are known to modify ethanol metabolism.[6] To our knowledge an experiment has never been reported in which chronic ethanol consumption is investigated in combination with different diets in experimental acute pancreatitis. In the present study the effect of long term ethanol consumption with different diets on experimental acute pancreatitis was studied in rats.

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Methods

ANIMALS

The total number of animals used in this study was 414 male Wistar rats. One hundred and ninety two of these animals were used as control animals for measuring haematocrit, calcium, and triglyceride levels. Acute pancreatitis was induced in 192 animals; the other 30 rats for the collection of bile, which was used for the induction of acute pancreatitis.

Animals of three months of age were used and these were randomly divided into eight different groups, each consisting of 24 animals. Two rats were housed in each cage and they were kept in an air conditioned room where temperature and artificial light were controlled (+20°C, 24 hours circadian cycle: 12 hours light, 12 hours dark). All animals had free access to food and drinking solution

according to their group. The rats used for bile donors were handled in the same way but were fed with standard laboratory food (Astra-Ewos, Sweden) and drank only tap water.

DIETS

Special diets used in this study were produced by Special Diet Services, Essex, Great Britain, apart from the standard diet mentioned above. The composition of the diets is shown in Table 1.

In our standard diet (S-diet) all fibre came from grain, which was the main source of carbohydrates as well in the form of starch. Other raw materials used for producing this diet were cereals, fish protein concentrate, roasted soya meal, fodder yeast and both animal and vegetable fat.

The fat rich diet (F-diet) was essentially a mixture of vegetable and animal proteins together with beef tallow and cereals. The fat contribution was provided by meat and heat treated soya bean mixed with beef tallow. Fibre was essentially cellulose. The protein content in the protein rich diet (P-diet) was achieved with casein, whole egg, and a heat treated soya bean concentrate. The fat content was achieved by the use of whole egg; fibre was essentially cellulose. The high carbohydrate level in carbohydrate rich diet (C-diet) was achieved by keeping protein low (6·8%) and utilising pure cornflour starch, although this diet also contained approximately 6·5% total sugars.

Essential trace elements and vitamins were added to all diets so that there were no other notable differences between the diets, except those presented in Table 1.

Table 1 *Percentage composition of the diets*

Constituent		*Standard*	*Fat rich*	*Protein rich*	*Carbohydrate rich*
Fat	%	5·40	35·00	9·50	2·00
Protein	%	24·10	22·00	75·00	6·80
Carbohydrate	%	48·60	7·00	2·20	80·20
Moisture	%	11·70	10·00	6·00	5·00
Fibre	%	3·90	20·00	2·00	0·00

Table 2 *Experimental groups and abbreviations used in the text*

Diet	*Abbrev*	*Drinking fluid*	*Abbrev*
Standard	S	Water	SW
		Alcohol	SA
Fat rich	F	Water	FW
		Alcohol	FA
Protein rich	P	Water	PW
		Alcohol	PA
Carbohydrate rich	C	Water	CW
		Alcohol	CA

EXPERIMENTAL DESIGN AND THE INDUCTION OF ACUTE PANCREATITIS

In each dietary group of 48 rats, 24 animals received 15% (v/v) ethanol in their drinking water for 12 weeks. The other 24 served as controls, receiving tap water. Abbreviations used for different groups are given in Table 2. Before the induction of acute pancreatitis all animals fasted 24 hours, obtaining tap water only; alcohol was also substituted with water.

Laparotomy was carried out under ether anaesthesia through a short midline incision. The pancreas was exposed and the common bile duct was cannulated with a thin blunt needle through the duodenum. The hepatic ducts were closed with a clamp at the liver hilus. Pancreatitis was induced by infusion of bile into the pancreatic duct with an automatic infusor (AG Braun, Melsunge, BRD), using a constant speed of 0·4 ml/min at a pressure just exceeding the penetration pressure of the pancreas.[7] The infused bile was collected under anaesthesia from the healthy rats. The infused volume of bile was adapted to the weight of the animal, the relation being 20 µl of bile/10 g of body weight. The operated animal was always weighed just before laparotomy and the puncture site was then carefully closed in two layers and the rats subsequently returned to their cages where they had free access to water. Twenty four hours after the induction of acute pancreatitis a relaparotomy was undertaken and the rats were killed by cannulating the abdominal aorta.

SAMPLING, EVALUATION OF GROSS FINDINGS AND HISTOLOGY

Necropsy was done on all animals that died. Ascites was collected during the second laparotomy if there was a measurable amount of it in the abdominal cavity.

The amount of macroscopic fat necrosis was graded by an arbitrary scale (0 to +++).

The pancreas was resected and the histological specimens were always taken from the same place, 2 cm proximal to the splenic hilus. The specimens were fixed in 10% buffered formalin, embedded in paraffin and the sections, 5 µm in thickness, were stained with haematoxylin and eosin (H & E). Three different morphological components were used in the evaluation of inflammation: the amount of inflammatory cells, haemorrhage and parenchymal cell necrosis. A general description of pancreatic inflammation was then evaluated on an arbitrary scale.[8]

ASSAYS

Haematocrit was determined with an Adams autocrit centrifuge. Calcium assay was carried out by the

routine method using a calcium titrator. Triglycerides were assayed with a neutral fat (triglycerides) test (Boehringer Mannheim GmbH; Cat. No. 15989). The amylase content of the plasma was measured with the Phadebas amylase test (Pharmacia).

STATISTICAL ANALYSIS

The significance of the differences between the groups were tested by one way analysis of variance continued by two tailed Bonferroni *t* test for non-paired values. The significance between the mortality rates were calculated using the Fisher's exact four-fold test and the differences between the gross findings in various groups with the χ^2 test.

Results

All animals tolerated the diet period of 12 weeks well. Death did not occur in any of the groups before or during the induction of acute pancreatitis. Individual follow up of food consumption was not possible, as special cages planned for this purpose were not available. Food consumption was, however, followed as groups and the food intake was diminished in all dietary groups by chronic ethanol ingestion and these animals also gained less weight than the corresponding controls receiving water. The changes in body weight were comparable with each other in different groups (Table 3), and no significant differences developed between the groups.

MORTALITY

The mortality rate in various groups is shown in Table 4. The highest mortality was observed in the FA, SA, and PA groups, revealing that ethanol consumption intensified acute pancreatitis in combination with all diets. The CA-group was, however, an exception: the lowest mortality was found in the groups receiving the C-diet. In this group the long term ethanol consumption did not increase the mortality rate significantly.

Table 4 *Mortality in different groups during the first 24 hours of acute pancreatitis*

Group	*n*	*Mortality*	*%*	*p**
SW	24	1	4·2	—
SA	24	7	29·2	<0·05
FW	24	3	12·5	NS
FA	24	9	37·5	<0·01
PW	24	4	16·7	NS
PA	24	5	20·8	NS
CW	24	0	0·0	NS
CA	24	1	4·2	NS

*The values of p were calculated with Fisher's exact four-fold test using the SW-group as the control group.
Abbreviations see for Table 2.

FAT NECROSIS

Laparotomy was done again 24 hours after the induction of acute pancreatitis. Table 5 shows the occurrence of fat necrosis in different groups. The most extensive fat necrosis was observed in the FA-group and the least in the CW-group. These differences were also statistically significant when compared with the SW-group (Table 4). The FA-group was the only one in which every animal had at least macroscopic fat necrosis on the surface of the pancreas.

HISTOLOGICAL STAGE OF INFLAMMATION

The most significant findings of acute inflammaton were found in the CA-group ($p<0·05$) with moder-

Table 3 *Body weights in different groups at the beginning and in the end of the diet period of 12 weeks*

Group	*n*	*Weight 1**	*Weight 2†*
SW	24	305±41	425±40
SA	24	349±60	428±30
FW	24	306±43	424±53
FA	24	317±33	330±36
PW	24	315±39	352±50
PA	24	352±24	345±42
CW	24	323±22	366±32
CA	24	350±22	353±26

*Weight 1=Mean±SD of the body weights at the beginning of the experiment.
†Weight 2=Mean±SD of the body weights after 12 weeks of diet period.
Abbreviations see for Table 2.

Table 5 *Fat necrosis in different groups*

Group	*n*	***	*†*	*‡*	*0*	*p§*
SW	23	4	3	11	5	—
SA	17	3	6	6	2	NS
FW	21	6	3	8	4	NS
FA	15	10	2	3	0	<0·05
PW	20	1	5	8	6	NS
PA	19	0	2	13	4	NS
CW	24	3	1	4	16	<0·01
CA	23	0	4	11	8	NS

0=no fat necrosis observed.
*=wide spread fat necrosis.
†=peripancreatic fat necrosis.
‡=fat necrosis limited on the surface of the pancreas.
§p calculated with χ^2 test using the SW-group as the control group.
Abbreviations see for Table 2.

ate or heavy inflammatory changes. In the PA-group the stage of inflammation was histologically slighter ($p<0.01$). No significant inflammatory changes were histologically found in the FA-group or any other experimental group. The complete results of histological evaluation will be presented elsewhere.[8]

PERITONEAL EXUDATE

Ascitic fluid was collected whenever possible. Table 6 presents the number of surviving animals in which a sufficient volume of peritoneal exudate was found. Ascites was frequently found in animals of the F- and SA-groups. In the P-groups it was less common, and in the CA-group it could not be collected at all.

HAEMATOCRIT

Haematocrit was determined in every animal (Table 7). The control values were obtained from the rats without acute pancreatitis. No significant differences were found between any of the control groups. No statistically significant differences were found either between the groups in which acute pancreatitis was induced. A tendency towards haemoconcentration during the first 24 hours after the induction of acute pancreatitis was noticed irrespective of diet. The increases were significant in the SA, FA, and PA-groups (Table 7).

Table 6 *Ascites formation among the survived animals after 24 hours of acute pancreatitis*

Group	*n*	*Ascites collected (n)**	*%*	*p†*
SW	23	6	26	—
SA	17	10	59	<0·05
FW	21	11	52	NS (<0·1)
FA	15	9	60	<0·05
PW	20	6	30	NS
PA	19	2	11	NS
CW	24	5	21	NS
CA	23	0	0	<0·05

*Number of animals where ascites was possible to collect more than 1 ml.
†p calculated with Fisher's exact probability and χ^2 tests using the SW-group as the control group.
Abbreviations see for Table 2.

Table 7 *Changes in haematocrits (Hcr) before and after 24 hours of acute pancreatitis in different groups*

Group	*n*	*Hcr (x±SD)*	*p*	*n*	*Hcr (x±SD)*
SW	24	0·46±0·02	NS	23	0·49±0·05
SA	24	0·46±0·03	*	17	0·52±0·08
FW	24	0·46±0·02	NS	21	0·50±0·04
FA	24	0·46±0·02	*	15	0·53±0·08
PW	24	0·48±0·02	NS	20	0·48±0·07
PA	24	0·47±0·02	*	19	0·52±0·04
CW	24	0·46±0·01	NS	24	0·47±0·06
CA	24	0·47±0·03	NS	23	0·48±0·02

*indicates $p<0.001$.
All differences among the control groups and AP-groups (vertical comparison) were statistically nonsignificant.
Abbreviations see for Table 2.

CALCIUM

The calcium concentration in plasma was determined from the control animals (2·49±0·13 mmol/l) and after 24 hours of acute pancreatitis (2·45±0·21 mmol/l). No significant differences were found between any of the control or AP-groups. A tendency of the plasma calcium concentration to decrease was noticed, however, in most of the AP-groups.

TRIGLYCERIDES

Among controls, the highest concentrations of plasma triglycerides were observed in the CA-groups (1·57±1·07 mmol/l) and the lowest in the SW-group (0·39±0·29 mmol/l). Chronic ethanol consumption increased triglyceride concentrations in all diet groups, but only in the FA-group (0·82±0·27 mmol/l) was the difference statistically significant in comparison with the SW-group.

AMYLASE

Serum amylase concentrations in the plasma were measured after 24 hours of acute pancreatitis. The highest concentrations were assayed in the PW- and PA-groups (13 213±1 0542 and 16 953±11 016 U/l, respectively) and the lowest in the FA-group (4283±1042 U/l).

Discussion

The ductal model was selected for the induction of pancreatitis as it is the most widely used in experimental studies concerning the pathogenesis of acute pancreatitis.[9] In the ductal model the nature and volume of injected material have an effect on the survival of the experimental animals.[7 10] Bile as an infusate is known to cause an oedematous acute pancreatitis associated with low mortality when injected into the pancreatic ducts, and therefore it was selected for this experiment to simulate bile reflux as closely as possible.[11] The volume and pressure were kept to a minimum to cause oedematous pancreatitis without ductal cell disruptions.[7 9]

Regarding the effect of different diets, the P- and F-diets increased the mortality rate, which was highest in the PW-group. This is in agreement with Maki's work.[4] Another report showed a divergent

result; namely that experimental acute pancreatitis was most severe in dogs that had been fed a high fat diet.[3] It is worth noting that although the induction methods and animals were different in these two papers, high fat and high protein diets intensified acute pancreatitis in each case.

Fat necrosis in this study was found to be the most extensive in the FA-group, among which the mortality rate was also the highest. No deaths occurred in the CW-group, and the incidence of fat necrosis was also the lowest. Fat necrosis was selected as one parameter in the criteria for numerical grading of acute pancreatitis in the study performed by Haig.[3] He did not table the results as systematically as in the present study, but fat necrosis was more common in the group of animals with severe acute pancreatitis The same observation was made by Maki *et al.*[4]

Haemodynamic effects were caused by peritoneal exudate formation during an attack of acute pancreatitis, not only because of its effect on the circulating blood volume but also because of toxic products in ascitic fluid.[12] Haemorrhagic ascitic fluid during acute pancreatitis has been shown to contain substances that may produce many systemic manifestations of acute pancreatitis – for example, renal failure[13] cardiovascular lesions,[14] hypocalcaemia (as mentioned above), hypotension and haemoconcentration.[15] Haemoconcentration has not been registered in the two previous reports on this subject.[3 4] In the present study, significant haemoconcentration was observed in the SA-, FA- and PA-groups, where the highest mortality was observed. There was a poor correlation between the mortality and the histological findings of the pancreas in our study.[8] Also this fact may suggest the importance of other factors for the survival of the animals.

Many factors, such as ethanolism and different diets,[16] are able to induce an increase in serum triglycerides. Thus it was necessary to determine if any of these diets alone or combined with ethanol caused hypertriglyceridaemia and so predispose the animals to acute pancreatitis. Hyperlipaemia has been proposed both as a cause of acute pancreatitis (primary) and a consequence (secondary).[16] Hyperlipaemia as a cause of acute pancreatitis has been questioned recently, however, by Stigendal and Olsson.[17] Their study provided no evidence of an important role for lipoprotein abnormalities in the development of human acute pancreatitis. In this experimental model hypertriglyceridaemia correlated well with the other findings only in the FA-group in the assessment of the severity of acute pancreatitis. The highest levels of triglycerides were determined in the CA-group, but mortality rate or other findings did not differ from the SW-group. It therefore seems that factors other than hypertriglyceridaemia are more important in the development of acute pancreatitis in this model.

It is concluded that diet has an effect on the mortality rate in acute pancreatitis, which is further increased by long term ethanol consumption; death was especially common with fat rich and protein rich diets. The increase in mortality rate seemed to be caused by the toxic substances in the peritoneal exudate, which was more obvious in the groups with a high incidence of death. Animals receiving the protein rich diet provided an exception, however. It could thus be possible that the diet alters the contents and increases the toxicity of the ascitic fluid. Even small amounts of peritoneal exudate may cause severe systemic effects that lead to death. This hypothesis might explain the beneficial effect of peritoneal lavage seen both in human and experimental studies[12] and individual susceptibility to acute inflammation of the pancreas.[1 3]

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