Changes of oxidative stress, inflammatory mediators and malnutrition in acute and chronic pancreatitis

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Ph.D. thesis

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1. INTRODUCTION AND AIMS

The acute pancreatitis is characterized by increased release of exocrine enzymes, local inflammatory process within the pancreas leading to systemic inflammation and increased catabolism. In 15-20% of cases, a severe, necrotizing form is seen, with mortality rate up to 40%.

Different experimental models of acute pancreatitis are useful tools for examination of factors playing role in the pathogenesis of acute pancreatitis. L-arginine excess has been used as a metabolic model of pancreatitis in rats, but less is known about the effect of another basic amino acid lysine. The comparison of secretory and morphological changes in these two models will help in understanding the mechanism of pancreatic destruction.

In acute pancreatitis increased intestinal permeability and bacterial translocation play a central role in development of life threatening septic complications. Enteral nutrition opened a new era in the prevention and treatment of acute pancreatitis. It decreased the number of late septic complications, rate of mortality and duration of hospital stay. Beside preventing gut atrophy and consequent bacterial translocation jejunal feeding might ameliorate the inflammatory response. Jejunal feeding has been successfully used in our department for more than 10 years. In our retrospective study patients with severe necrotising pancreatitis feed enterally had less complications, surgical interventions and lower mortality, compared to the parenteral group. Therapeutic effect of jejunal nutrition may originate from preserved gut motility and secretory functions, optimal energetic and nutritional characteristics. In acute pancreatitis because of increased catabolism and proteolysis there is a high energy demand, thus energy and protein intake is an important part of the treatment.

The presence of reactive oxygen species in acute pancreatitis have been shown in numerous experimental and clinical studies, but the exact role of these radicals is not well known. Early release of free radicals have been implicated in the pathogenesis, and the oxidative stress showed significant correlation with the severity of disease. The involvement of oxygen radicals is not fully elucidated in different forms of chronic pancreatitis. Clinical studies revealed that these patients are often deficient in several antioxidants (glutathion, vitamin A, E and C), and showed increased lipid peroxidation products in the pancreatic tissue obtained after resective procedures.

Exact cause of acute pancreatitis remains unknown, therefore the therapeutic goals are directed toward modulation of hyperinflammatory state in the first phase of the disease.
Potential administration of immunonutrients might be of benefit through immunomodulation and restoring gut integrity. Dietary n-3 polyunsaturated fatty acids (PUFAs) can reduce severity of inflammatory injury by altering the availability of arachidonic acid in tissue phospholipids and modulating eicosanoid synthesis.

1.2. Aims
1.2.1. Comparing the pancreatic damage caused by excess of two basic amino acids in the experimental part, we examined:
   - which factors play role in the development of biochemical, histological and electron microscopic changes.
1.2.2. During clinical examinations, in patients with acute pancreatitis receiving early enteral nutrition we studied:
   - the correlation between early jejunal feeding and inflammatory mediator release, oxidative stress in the first and late phases of the disease,
   - the frequency of antioxidant depletion during the treatment, the correlation between the decreased antioxidant capacity and other indicators of severity of inflammation,
   - the changes of nutritional and protein status of patients during enteral nutrition, the effect of PEM on the course of the disease and development of complications,
   - the effect of enterally supplemented n-3 PUFAs on inflammation, reactive oxygen radical release and outcome.
1.2.3. The antioxidant and nutritional status were also measured in patient suffering from chronic pancreatitis.
1.2.4. Based on our results we summarized the principles of dietary treatment of patients with acute and chronic pancreatitis.

2. EXPERIMENTAL PART
The effect of amino acids – pancreatic damage caused by excess lysine and arginine in rats

2.1. Methods:
Animals: Male Wistar rats weighing 170-240g were divided into three groups. In group I (n=32) acute pancreatitis was induced by intraperitoneal injection of 20% solution of L-lysine (350 mg /100g bw), the group II (n=20) was treated with 20% solution of L-arginine (500 mg/100g bw), the group III (n=20) received equal volumes of saline.
Methods: After sacrificing the animals, blood sampling and excision of the pancreas we measured serum amylase activity and pancreatic protein, amylase, trypsin and lipase contents at 12 h – 24 h – 48 h – 7 days after the induction.

2.2. Summary of results:
A direct non-specific membrane damaging and cell-necrotizing effects were seen not only after arginine, but also after lysine administration. Administration of excess lysine induced more rapid and severe damage to the pancreas with significant early and late mortality. The histological and electron microscopic changes showed great similarity, in both groups the changes were characterized with intense tissue oedema and cytoplasmatic vacuole formation, appearance of autophagic granules and a decrease in the number of zymogen granules. In the case of both model loss of membrane integrity of acinar cells, derangement of endoplasmatic reticulum and structural changes of mitochondria were seen, and changes typical for cellular necrosis developed. Non-invasive model of necrotizing pancreatitis induced by excess lysine and arginine may be useful for investigating different pathogenetic factors playing role in the development of acute pancreatitis.

3. CLINICAL INVESTIGATIONS

In the first part of our investigations we studied cytokine release and changes of antioxidant status during enteral nutrition in patients suffering from acute pancreatitis (n=15).
In the next part we examined the antioxidant and protein status in patients with acute pancreatitis (n=50). The measurement of antioxidant capacity, protein metabolism and nutritional status were done also in patients suffering from chronic form of pancreatitis (n=50).
We studied the effect of enterally supplemented n-3 PUFAs (3.3 g/day) in moderate and severe form of acute pancreatitis. In a prospective, randomized clinical trial we compared the antioxidant status, extent of inflammation, the clinical course and complications in an n-3 PUFA treated group (n=14) and in patients receiving standard polymeric formula alone (n=14).
Patients and methods

Acute pancreatitis

The patients enrolled into the study were treated at the Department of Gastroenterology of MÁV Hospital. After admission the patients were classified into groups of severe and moderate pancreatitis according to the Atlanta classification system. At the first day of the treatment a naso-jejunal feeding tube of 10 F size was endoscopically placed into the second loop of jejunum behind the ligament of Treitz. Jejunal feeding was started with half strength, polymeric formula and energy intake was gradually increased to 1500-2000 kcal/day on the 2\textsuperscript{nd}-3\textsuperscript{rd} day of treatment.

Chronic pancreatitis

In patients with chronic pancreatitis we made the same examinations during follow-up. The diagnosis was earlier established by functional tests (Lundh and Lipiodol tests), endoscopic retrograde cholangiopancreatography and radiological examinations.

3.1. Changes of cytokine release and antioxidant status in acute pancreatitis during enteral nutrition

We studied 15 patients (mean age:54.0 range:34-83 years) with acute pancreatitis (etiology: alcoholic 6, biliary 3, unknown origin 3). Severe necrotizing pancreatitis developed in 3 patients, in two cases milder form of pancreatitis was accompanied with severe cholangitis. We measured serum C-reactive protein (CRP), tumornecrosis factor alfa (TNFa) and interleukin 6 (IL-6) levels, the total antioxidant status (TAS), superoxide-dysmutase (SOD) and gluthation-peroxidase (GPOX) activity in the erythrocytes. Antropometry (weight, height, middle arm circumference, triceps skinfold measurements), determination of serum acute phase proteins and total lymphocyte count were used for evaluation of nutritional status during the treatment.

3.2. Antioxidant status and protein-energy malnutrition in acute and chronic pancreatitis

In the second part of our investigations 50 patients with acute pancreatitis (mean age:54,3, range:21-5 years, 10 severe and 40 moderate) and 50 patients with chronic pancreatitis (mean age:58,2, range:19-72 years) were enrolled to the study, 20 age and sex matched healthy adults served as controls. We measured the erythrocytes SOD activity and serum TAS at admission, day 3, 7 and 14. At the same time nutritional status, degree of malnutrition was
determined during the treatment by anthropometry and measurements of biochemical markers.

3.3. Effect of enterally supplemented n-3 PUFAs in acute pancreatitis

In a prospective, randomized clinical trial from 28 patients with moderate or severe acute pancreatitis, 14 (mean age: 54.6, range: 33-83 years) were randomized to receive n-3 polyunsaturated fatty acids (n-3 PUFAs: 1.66 g eicosapentaenoic acid, EPA and 1.19 g docosahexaenoic acid, DHA). The patients were supplemented enterally, through the jejunal tube with a daily dose of 3.3 g n-3 PUFAs (fish oil) divided into 3 doses, for 7 days. The control, not supplemented group (n=14, mean age: 52.9, range: 27-78 years) received isocaloric polymeric enteral formula alone. Measurement of erythrocyte SOD activity, serum total antioxidant status (TAS), vitamin A and E, CRP and transthyretin concentrations were performed at admission, day 3, 7 and 14 of the treatment. We measured the serum total cholesterol, triglyceride and fatty acid levels of patients at day 1 of treatment and at day 7 after the supplementation with fish oil. The primary outcome variables were the time receiving jejunal feeding, the length of hospital stay and the development of pre-defined complications.

4. RESULTS AND DISCUSSION
4.1. Changes of cytokine release and antioxidant status in acute pancreatitis during enteral nutrition

The serum IL-6 levels were already increased at the time of admission in 14/15 cases, they reach the peak level after 24-48 hours, while there were no changes seen in the TNF-a levels. In severe forms the decrease in IL-6 levels was slower during the treatment compared to moderate pancreatitis. The IL-6 levels were increased in two cases of moderate pancreatitis complicated with severe cholangitis. Our results suggest that there is a pronounced cytokine release in severe cases inspite of early enteral nutrition.

The SOD activities were significantly lower in severe acute pancreatitis and in cases accompanied with severe cholangitis, compared to moderate forms. It showed significant increase after 14 days of treatment compared to admission. The serum TAS was in a low normal reference range at admission and it was significantly decreased after the first and second week of treatment. Gluthation peroxidase activity was in a normal reference range during the hospital stay, but it was significantly lower at day 7 compared to admission,
perhaps because of increased lipid peroxidation. According to our preliminary results we continue with the examination of antioxidant defense system in patients with acute pancreatitis.

4.2. Antioxidant status and protein-energy malnutrition in acute and chronic pancreatitis

Our results demonstrated that patients with acute pancreatitis suffer from severe depletion of antioxidant not only at the time of admission, but during the hospital treatment. At admission the SOD activity was already significantly lower compared to controls, and remained significantly decreased, as the serum TAS at the end of the first and second week of the treatment. Decreased SOD activity showed significant correlation with the severity of disease and APACHE II scores at admission. In acute pancreatitis it is essential to identify at an early stage those patients who will develop severe necrotising pancreatitis without effective treatment. Decrease in SOD activity seems to be a useful indicator of ongoing pancreatic and systemic inflammation, and for predicting outcome in these patients. Sustained generation of reactive oxygen species and prolonged depletion of antioxidant defense system predispose to development of late complications. Early jejunal feeding with supplementation of antioxidants could be an important part of the treatment, as it was shown in experimental studies.

The mean duration of hospital stay was 18.2+9.13 days, and the patients received jejunal nutrition for 14.3+7.87 days. In patients with acute pancreatitis determination of prealbumin and albumin concentrations seems to be a good indicator of nutritional status and protein malnutrition. Decreased concentration of prealbumin at admission predicted the severity of pancreatitis and catabolic stress. Jejunal nutrition prevented weight loss in patients with acute pancreatitis and the deterioration of protein-energy malnutrition.

Significantly reduced SOD activity was found in patients with chronic pancreatitis compared to the control group, however, this decrease was milder in comparison to acute forms. In this group 12/50 (24%) of patients had moderate or high risk of PEM. Patients with alcohol-related chronic pancreatitis had significantly decreased mean prealbumin levels compared to pancreatitis of other origin. Also, the incidence of protein malnutrition and that of severe, complicated acute exacerbation was higher in these patients.
4.3. Effect of enterally supplemented n-3 PUFAs in acute pancreatitis

First in the literature, we enterally supplemented n-3 PUFAs (EPA+DHA) in a prospective, randomized clinical trial. Enhanced dietary intake of EPA and DHA resulted not only in a significant increase of the contribution of n-3 PUFA to the fatty acid composition of serum lipids but in a significant decrease of the availability of arachidonic acid. The n-3 to n-6 PUFA ratios increased significantly in serum lipids of patients receiving n-3 PUFA supplementation, whereas remained unchanged in the controls. As a result of the treatment the length of jejunal feeding and hospital stay was significantly reduced. Inspite of the small number of patients, our results suggest that early supplementation with n-3 fatty acids in acute pancreatitis might attenuate the inflammatory processes in the first phase of disease. Enteral supplementation of n-3 fatty acids did not cause pancreatic stimulation, the absorption from the gastrointestinal system seems to be good according to serum fatty acid analysis. With a supplementation of vitamin E the oxidation of polyunsaturated fatty acids was prevented. The use of an enteral formula supplemented with n-3 fatty acids and with different antioxidants might help to reduced inflammation and prevent complications during the treatment of acute pancreatitis.

5.1. DISCUSSION OF NEW RESULTS

- In acute pancreatitis there is an increased risk of oxidative stress, a reduction in antioxidant capacity of patients was found already at admission and during the first two weeks of treatment. Depletion of antioxidant defense system predispose to development of late complications. As part of the supportive therapy enteral supplementation with some natural antioxidants (vitamin C and E, selenium) is recommended.
- In acute pancreatitis jejunal nutrition prevented the deterioration of acute PEM and increased weight loss.
- Determination of SOD activity and serum prealbumin concentration could be a useful indicator of disease severity.
- The n-3 to n-6 PUFA ratio increased in serum lipids of patients with acute pancreatitis after enteral supplementation with n-3 fatty acids, EPA and DHA. The length of jejunal feeding and hospital stay was significantly reduced. Optimal fatty acid composition of enteral formulas used in patients with acute pancreatitis should be further investigated in large-scale, dose-effect clinical trials.
- In chronic pancreatitis in 12/50 (24%) of patients moderate or high risk of PEM was found, and the incidence of protein malnutrition was higher in patients with alcohol-related forms. Determination of nutritional status and risk of malnutrition, dietary supplementation of antioxidants might lead to better quality of life in chronic pancreatitis.

PUBLICATIONS

Publication in connection to the thesis


Published abstracts of presentations


