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Local and systemic effects of aging on acute pancreatitis

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ABSTRACT

Background: /Objectives: Evaluation of the local and systemic effects of aging on the severity of acute pancreatitis (AP) in an experimental rat model in elderly animals.
Methods: AP was induced in Wistar rats by intraductal 2.5% taurocholate injection and divided into two groups: Young (3 month old) and Aged (18 month old). Two and 24 h after AP induction blood samples were collected for determinations of amylase, AST, ALT, urea, creatinine, glucose, and of plasma I-FABP. TNF-α and IL-6 levels were determined in serum and ascitic fluid. Liver mitochondrial function and malondialdehyde (MDA) contents, pancreas histological analysis, and pulmonar myeloperoxidade (MPO) activity were performed. Bacterial translocation was evaluated by bacterial cultures of pancreas.
Results: A significant increase in serum amylase, AST, ALT, urea, creatinine, glucose, I-FABP, and IL-6 levels, and a reduction in serum and ascitic fluid TNF-α levels were observed in the aged group compared to the young group. Liver mitochondrial dysfunction, MDA contents, and pulmonary MPO activity were increased in the Aged AP group compared to the Young AP group. Positive bacterial cultures obtained from pancreas tissue in aged group were significantly increased compared to the young group. Acinar necrosis was also increased in aged AP group when compared to young AP group.
Conclusion: Aging worsens the course of acute pancreatitis evidenced by increased local and systemic lesions and increased bacterial translocation.

Introduction

Acute severe pancreatitis (AP) is associated with a high morbidity and mortality mainly in the elderly patients [1–3]. Some studies have associated the increased morbidity and mortality to the presence of comorbidities in the elderly population [2] however other reports consider advanced age to be an independent prognostic factor in AP [4]. The mechanism that puts the aged pancreas, and aged patient, at risk for severity in pancreatitis is still unknown. Besides the presence of comorbidities [2], impairment of immune response to the injury, a reduction of pancreatic protective mechanism [5], increased systemic inflammation and thrombosis [6] or an increased intestinal damage with increased bacterial translocation [7] may result in distant organ damage with increased morbidity and mortality. In a previous report from our laboratory it was demonstrated an increased intestinal inflammation in aged animals with AP that may contribute to intestinal barrier dysfunction, bacterial translocation and distant organ damage [7]. However there are few studies evaluating simultaneously local and systemic effects of ageing in AP. The aim of this study was not only to evaluate the local and systemic effects of aging on acute pancreatitis but also to established the possible etiology of aged related vulnerability in AP.

Methods

Animals

Male Wistar rats were housed in individual cages and kept under standard conditions (12 h of light/dark cycle and temperatures between 22 and 24 °C) with free access to a standard chow and water. Rats were then randomly divided into two groups: *Young* (n = 24) 3 month old rats (230–270 g body weight) and *Aged* (n = 24) 18 month old rats (600–700 g body weight) [5]. Each group had ten sham-operated animals as controls.

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The experimental protocol was approved by the Ethics Committee for Animal Research from University of Sao Paulo School of Medicine. Animals received care in accordance with the Guide for the Care and Use of Laboratory Animals.

Induction of AP and experimental design

Surgical anesthesia was induced with ketamine chloride 50 mg/ml (0.2 ml/100 g body weight) (Ketalar, Park-Davis, São Paulo, Brazil). AP was induced in anesthetized rats by retrograde injection of 0.5 ml of 2.5% (w/v) sodium taurocholate (Sigma Chemical Company TM, St Louis, MO, USA) in 0.9% (w/v) NaCl into the main pancreatic duct for 1 min at a constant rate using an infusion pump (KD Scientific, Holliston, MA). A clamp was applied across the proximal hepatic duct during the injection [8,9]. Animals were allowed to recover after induction of pancreatitis. Since the rat pancreas weight does not increase with age the same amount of sodium taurocholate was used in both young and elderly rats.

The animals were then divided into the following groups: Young AP group: 14 animals submitted to AP induction (2 and 24 h) and Old AP group: 14 animals submitted to AP induction (2 and 24 h). Each group had ten sham-operated animals as controls: Young Sham (n = 10) and Old Sham (n = 10) groups where animals were submitted to the operative procedure without induction of AP.

At two and 24 h after AP induction, animals were re-anesthetized and serum samples collected for determinations of amylase [10], AST, ALT, urea, creatinine, glucose and ileal fatty acid binding protein (I-FABP). TNF-α, IL-6 and IL-10 levels were determined in serum. Ascitic fluid samples were assayed for amylase, TNF-α, IL-6 and PGE-2 levels. Ascitic fluid volume was also evaluated. Liver mitochondrial oxidation and phosphorylation, liver malondialdehyde (MDA) contents and pulmonary myeloperoxidase (MPO) activity were also assessed. Bacterial translocation was evaluated by bacterial cultures of pancreatic tissue collected under aseptic conditions. A portion of the pancreatic tissue was fixed in 10% buffered formalin for histological analysis.

Biochemical analysis

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, glucose was assayed by the optimized ultraviolet method (COBAS C111) from Roche (Roche Diagnostics, Rotkrenz, Switzerland).

Determination of inflammatory mediators

TNF-α, IL-6 and IL-10 levels were measured in serum and in the ascitic fluid by a solid-phase sandwich enzyme-linked immune absorbent assay (ELISA) using commercial kits (Invitrogen, CA, USA). PGE-2 in the ascitic fluid was determined by ELISA using Cayman Chemical kit (MI, USA).

Liver mitochondrial oxidation and phosphorylation activities

Liver mitochondria were prepared as previously described [11]. Briefly, rat livers were rapidly excised and placed in medium containing 250 mM sucrose, 10 mM Tris-HCl, and 1 mM EGTA, pH 7.3, at 4 °C. The tissue was scissor-minced and homogenized in ice using a Teflon Potter homogenizer. The homogenate was centrifuged at 600 g for 10min. The supernatant was centrifuged for 10 min at 10,000 g to obtain the mitochondrial pellet. Mitochondrial suspension containing 30–40 mg/ml of mitochondrial protein was prepared and stored on ice before the assay of mitochondrial respiration.

The mitochondrial oxygen consumption was polarographically [12] measured using a Gilson 5/6H Oxygraph (Gilson Medical Eletronics, Inc., Middleton, WI) in a closed reaction vessel fitted with a Clark oxygen electrode (Yellow Springs Instruments Co., Yellow Springs, OH) at 28 °C. The incubation medium consisted of 120 mM KCl, 2 mM sodium phosphate, 10 μM rotenone, and 1 mM EGTA (Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetra-acetic acid), and was buffered at pH 7.3 with 5 mM Tris-HCl. Mitochondria were energized with potassium succinate as substrate at a final concentration of 10 mM. After a brief equilibration period, state 3 (activated state, S3) respiration was induced by the addition of 280 nmol adenosine diphosphate (ADP). The added ADP was phosphorylated to adenosine triphosphate (ATP) and the state 4 (basal state, S4) respiration was then measured. The oxygen consumption ratio in the presence of ADP to that in absence (respiratory control rate, RCR) was calculated as index of mitochondrial oxidation and phosphorylation activity [13].

RCR = oxygen consumption in the S3/ oxygen consumption in the S4

S3 and S4 were measured and reported as nmol oxygen per milligram mitochondrial protein per minute. Mitochondria protein content was determined by the method of Lowry et al. [14].

Lipid peroxidation analysis

Malondialdehyde (MDA) formation was used as indicative of lipid peroxidation occurrence in the tissues and was estimated as thiobarbituric acid-reactive substances (TBARS). Liver tissue (100 mg/ml) was homogenized in 1.15% KCl buffer, and centrifuged at 14,000 g for 20min. An aliquot of the supernatant was then added to a reaction mixture consisting of 1.5 ml 0.8% thiobarbituric acid, 200 μl 8.1% (v/v) sodium dodecyl sulfate, 1.5 ml 20% acetic acid (pH 3.5), and 600 μl distilled water. The mixture was then heated at 90 °C for 45min. After cooling to room temperature, samples were cleared by centrifugation (10,000 g for 10min), and the absorbance measured at 532 nm using malondialdehyde bis (dimethyl acetal) as external standard. The content of lipid peroxides was expressed as nmol MDA per mg of protein [15].

Pulmonary myeloperoxidase (MPO) activity

The presence of neutrophils in lung tissue was assessed by MPO activity. At the time of sacrifice, after blood sampling, lungs were perfused with 30–50 mL of 0.9% NaCl at 10 mL/min, using a syringe pump (model 975) from Harvard Apparatus, and fragments were harvested for analysis of mieloperoxidase (MPO) activity. Samples of 300 mg wet lung tissue were homogenized with a polytron homogenizer (Polytron PT-2100 homogenizer, Kinematica AG, Luzern, Switzerland) for 60 s in 1 ml of sodium phosphate buffer, pH 6.2, containing 0.5 g/dL hexadecyltrimethyl ammonium bromide and 5 mM of ethylenediaminetetraacetic acid. Homogenized samples were then sonicated at 40 Hz for 60 s, and centrifuged at 3,000 g for 30 min at 4 °C. MPO activity in the supernatant was assayed by measuring the change in absorption at 460 nm (A_{460}) resulting from the metabolism of hydrogen peroxide in the presence of *O*-dianisidine [16,17]. MPO content was expressed as units of MPO activity per gram tissue.

Ileal fatty acid binding protein (I-FABP)

In order to evaluate gut epithelial cell injury secondary to AP levels of I-FABP were measurement in plasma [18,19] by a solid-phase sandwich enzyme-linked immune absorbent assay (ELISA) using commercial kits (HyCult Biothechnology, Udenm, The

Table 1
Effect of aging in biochemical analysis on AP.

	2 h after A Young	2 h after AP Aged	24 h after AP Young	24 h after AP Aged
Ascitic fluid amylase (U/mL)	123 ± 5	209 ± 17 *	ND	ND
Serum Amylase (U/mL)	14.0 ± 0.8	19.0 ± 1.9 *	11.5 ± 0.9	13.8 ± 3.0 *
AST (U/L)	412 ± 69	603 ± 46 *	431 ± 39	742 ± 41 *
ALT (U/L)	106 ± 22	210 ± 27 *	134 ± 20	247 ± 45 *
Urea (mg/dL)	59 ± 3	83 ± 8 *	40 ± 3	81 ± 16 *
Creatinine (mg/dL)	0.3 ± 0	1.0 ± 0.1 *	0.4 ± 0	0.8 ± 0.2 *
Glucose (mg/dL)	153 ± 1.2	235 ± 12 *	147 ± 15	139 ± 12

Rats were divided into 2 groups: Young: 3 months old rats and Aged: 18 month old rats. Experimental acute pancreatitis (AP) was induced in Wistar rats by injection of 2.5% sodium taurocholate. Animals were sacrificed and analyzed two and 24 after the induction AP. Ascitic fluid and serum amylase activity, serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, and glucose were determined 2 h or 24 h after AP induction. Non-detectable (ND). Data are expressed as mean ± SEM. *p < 0.05 young compared to aged animals.

Netherlands).

Pancreatic infection

Twenty-four hours after AP induction five rats from each group were submitted to a midline laparotomy incision under anesthesia and pancreatic tissue was collected under aseptic conditions. Samples were weighed and homogenized and aerobic cultures were made using blood agar plates and MacConkey agar plates.

Pancreatic infection was evaluated by bacterial cultures of pancreas and expressed in colony-forming units (CFU) per gram. The presence of pancreatic infection was accepted when CFU/g was>10^5 [20].

Histological analysis of the pancreas

Pancreatic tissue was harvested at two and 24 h after AP induction, fixed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for light microscopy. Histological evaluation of the pancreas sections was performed by the same pathologist in a blinded way. The severity of acinar and fat necrosis was analyzed in accordance with Schmidt et a [21]. A scale of 0–4 was used according to the following histological scoring criteria:

Acinar necrosis

0 Absent
0.5 Focal occurrence of 1–4 necrotic cells/high-power field (HPF)
1 Diffuse occurrence of 1–4 necrotic cells/HPF
1.5 Same as 1 + focal occurrence of 5–10 necrotic cells/HPF
2 Diffuse occurrence of 5–10 necrotic cells/HPF
2.5 Same as 2 + focal occurrence of 11–16 necrotic cells/HPF
3 Diffuse occurrence of 11–16 necrotic cells/HPF (foci of confluent necrosis)
3.5 Same as 3 + focal occurrence of more than 16 necrotic cells/HPF
4 More than 16 necrotic cells/HPF (extensive confluent necrosis)

Fat necrosis

0 Absent
0.5 1 focus
1 2 foci
1.5 3 foci
2 4 foci
2.5 5 foci
3 6foci3.5 7 foci
4 8 foci or more

Statistical analysis

Continuous variables (amylase, AST, ALT, urea, creatinine, glucose, TNF-α, IL-6, IL-10, PGE2, liver mitochondrial function, liver MDA content, and pulmonary MPO activity between groups were compared using unpaired Student's *t*-test. Histological analysis, I-FABP and pancreatic infection were performed by the Mann-Whitney test. The data are expressed as mean ± SEM. Linear regression analysis was performed with use of the Spearman correlation coefficient. The level of $p < 0.05$ was considered statistically significant. The GraphPad Prism Software (GraphPad Software, SanDiego, CA) was used for statistical analysis.

Results

Effect of aging on biochemical data

Ascitic and serum amylase activity, AST, ALT, urea, creatinine and glucose were significantly elevated in all groups with AP (young and aged). However, it was observed a significant increase in the aged group compared with the young group with AP (Table 1). The results of the biochemical determinations of animals from the control and sham-operated groups are shown in Table 2. Serum

Table 2
Effect of aging in biochemical analysis in control and sham-operated animals.

	Control Young	Control Aged	Sham Young	Sham Aged
Ascitic fluid amylase (U/mL)	ND	ND	ND	ND
Serum Amylase (U/mL)	8.8 ± 0.5	7.4 ± 0.5	8.7 ± 0.6	9.1 ± 1.2
AST (U/L)	213 ± 5	164 ± 21	256 ± 24	286 ± 38
ALT (U/L)	58 ± 3	44 ± 2	65 ± 5	83 ± 14
Urea (mg/dL)	30 ± 2	57 ± 2 *	44 ± 3	54 ± 36
Creatinine (mg/dL)	0.3 ± 0	0.6 ± 0.1*	0.4 ± 0	0.5 ± 0.1
Glucose (mg/dL)	155 ± 5	167 ± 16	163 ± 6	166 ± 6

Rats Control and Sham-operated were divided into 2 groups: Young: 3 months old rats and Aged: 18 month old rats. Ascitic fluid and serum amylase activity, serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, and glucose were determined. Non-detectable (ND). Data are expressed as mean ± SEM. *p < 0.05 young compared to aged animals.

urea and creatinina were already elevated in aging control animals when compared to young.

Effect of aging on ascitic fluid and serum levels of TNF-α, IL-6 and IL-10.

After 2 h of AP induction ascitic fluid volume was similar in young (5.0 ± 0.6 ml) and in elderly animals (4.9 ± 0.4 ml). There was no ascitic fluid in the animals of the control and sham groups or in the animals submitted to AP in the 24-h observation period.

There was a significant reduction of TNF-α levels and an increase of IL-6 in ascites and serum at 2 h after AP in aged animals compared to the young group (Fig. 1A and B). Serum IL-10 levels were similar in both groups (Fig. 1C). No differences were found in PGE-2 in ascitic fluid between young and aged groups at 2 h after AP induction ($p = 0.962$).

Serum cytokines were not detected in young and old animals of the control groups, sham-operated and 24 h prior to induction of AP.

Effect of aging on liver mitochondrial function

Two hours after AP a transient liver mitochondrial dysfunction occurred in young animals compared to aged animals, mainly due to uncoupling of oxidative phosphorylation. There was an increase in the oxygen consumption rate by liver mitochondria in state 3 (S3) and state 4 (S4) respiration and a decrease in RCR. This liver dysfunction was recovered and did not occur in the sham group of young animals.

However, in aged animals 2 h after AP there was a liver mitochondrial dysfunction compared to young animals, demonstrated by a significant reduction in RCR ratio (Aged: 1.99 ± 0.08 *vs* Young: 2.44 ± 0.15, $p = 0.017$) and in S3 respiration (Aged: 47.93 ± 5.15 *vs* Young: 76.70 ± 6.09 nmolO_2/mg protein, $p = 0.005$). This dysfunction was also noted in sham aged animals (RCR: 2.34 ± 0.17 and S3: 52.65 ± 7.61 nmolO_2/mg protein), suggesting a previous degenerative process similar to that found in cellular ischemia (Fig. 2A, B and 2C).

Effect of aging on liver lipid peroxidation analysis

MDA liver content used as measure of lipid peroxidation in the organ was increased in young animals 2 h after AP (3.66 ± 0.22 nm MDA/mg prot) in comparison to the sham young group (1.61 ± 0.35 nm MDA/mg prot) ($p < 0.05$). The aged sham animals showed a higher MDA content (3.02 ± 0.27 nm MDA/mg prot) when compared to the young sham animals (1.61 ± 0.35 nm MDA/mg prot) ($p = 0.009$), but there was no additional increase in MDA content in the aged AP group (2.56 ± 0.23 nm MDA/mg prot) compared to aged sham animals (3.02 ± 0.27 nm MDA/mg prot) ($p > 0.05$) (Fig. 3).

Effect of aging on pulmonary myeloperoxidase (MPO) activity

We did not observe a significant difference of pulmonary MPO between control and sham-operated animals of young and old animals (Young control: 0.069 ± 0.013 DO 460 nm and young sham-opereted: 0.062 ± 0.002 DO 460 nm) (Aged control: 0.073 ± 0.007 DO 460 nm and aged sham-operated: 0.097 ± 0.024 DO 460 nm).

At 2 h and 24 h after induction of AP MPO activity was increased in the pulmonary tissue in both groups with AP compared to control and sham-operated animals. However, it was observed a significant increase in aged group compared to the young group with AP (Fig. 4).

Effect of aging on ileal fatty acid binding protein (I-FABP)

A significant increase in plasma I-FABP levels 24 after AP induction was observed in the aged group compared to the young group. In the animals of the sham groups there was no increase in plasma I-FABP levels (Fig. 5A).

We did not observe plasma I-FABP levels 2 h after AP induction levels and in control and sham-operated animals.

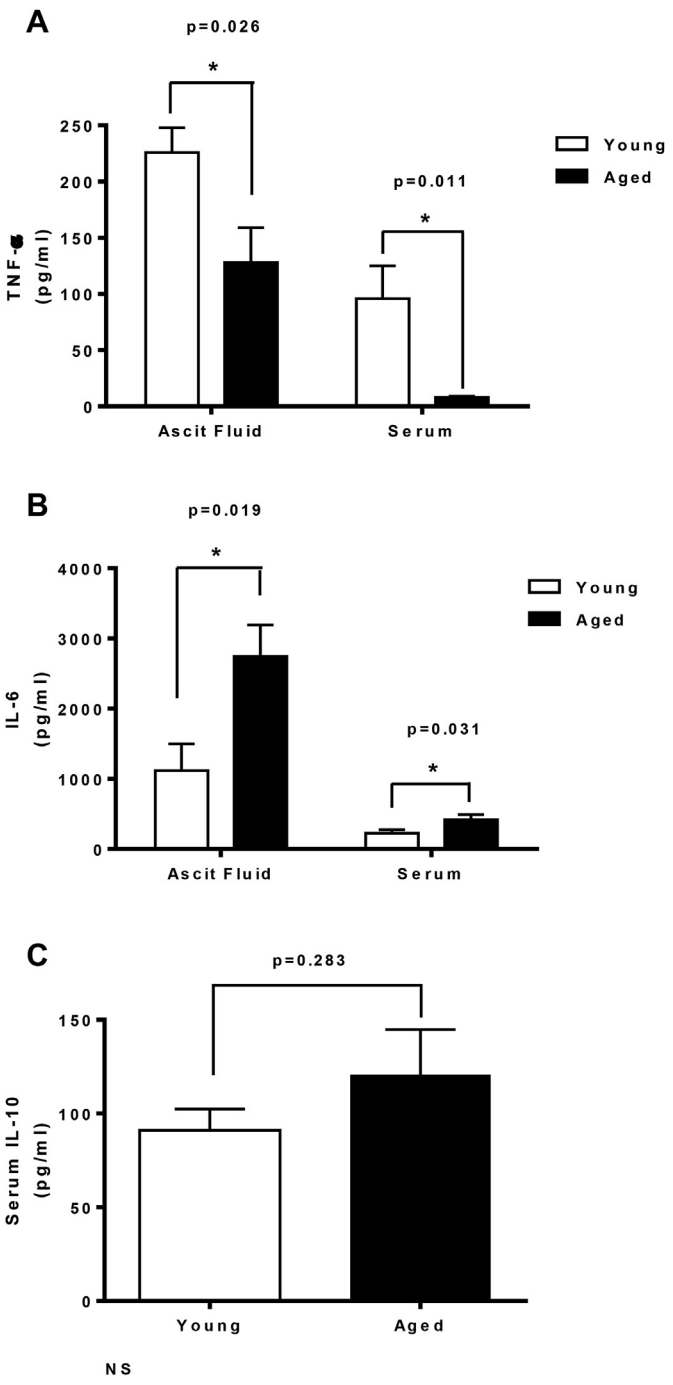

Fig. 1. Effect of aging on ascitic fluid and serum levels of TNF-α, IL-6 and IL-10. Groups of animals Young and Aged were submitted to acute pancreatitis (AP). Ascitic fluid and serum levels of TNF-α **(A)**; IL-6; **(B)** Serum IL-10 **(C)**, Data are expressed as mean ± SEM. $^*p < 0.05$.

Effect of aging on pancreatic infection

A significant increase in positive bacterial cultures obtained

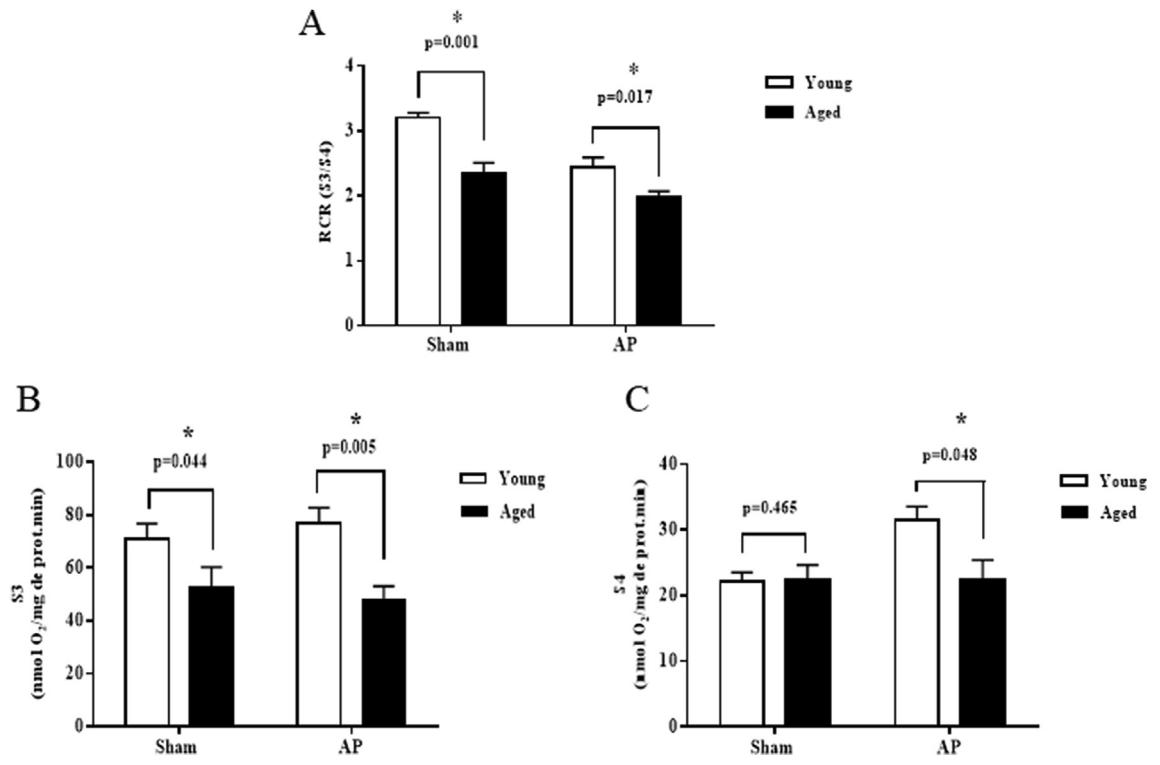

Fig. 2. Effect of aging on liver mitochondrial oxidation and phosphorylation activities. Groups of animals Young and Aged were submitted to acute pancreatitis (AP). **(A):** Respiratory control rate (RCR), **(B):** State 3 respiration (S3), **(C):** State 4 respiration (S4). Data are expressed as mean ± SEM. $^{*}p < 0.05$.

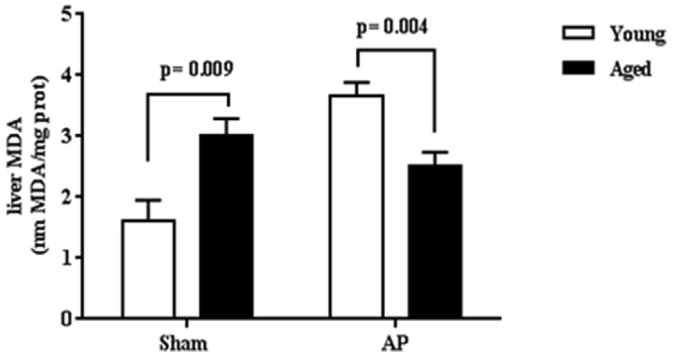

Fig. 3. Effect of aging on liver lipid peroxidation analysis. Groups of animals Young and Aged were submitted to acute pancreatitis (AP). Data are expressed as mean ± SEM * $p < 0.05$.

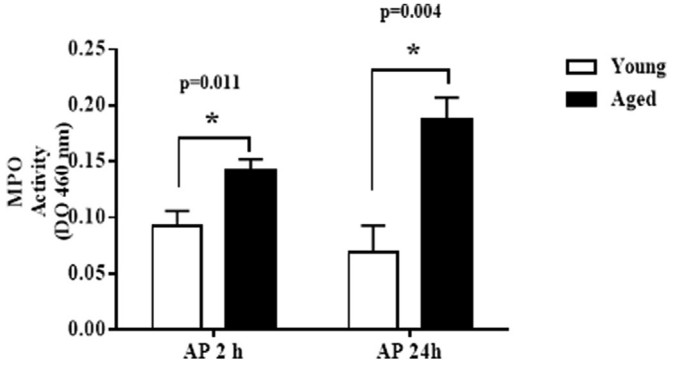

Fig. 4. Effect of aging on pulmonary myeloperoxidase (MPO) activity. Groups of animals Young and Aged were submitted to acute pancreatitis (AP). Data are expressed as mean ± SEM. $^{*}p < 0.05$.

from pancreas tissues in aged group was observed compared to young rats (Fig. 5B). In the animals 2 h after AP induction, control and sham groups no pancreatic infection was detected.

Correlation between the plasma I-FABP levels and pancreatic infection

A significant positive correlation was found between the plasma I-FABP levels and the pancreatic infection ($r = 0.888$, $p = 0.0004$) (Fig. 6).

Effect of aging on histological analysis of the pancreas

Histological evaluation of pancreatic tissue demonstrated a significant increase in steatonecrosis in the aged group at 2 h after AP induction (aged: 1.1 *vs* young: 0.3, $p = 0.004$) and 24 h (aged: 1.7 *vs* young: 0.1, $p = 0.015$). Increased acinar necrosis was also observed in aged group at 24 h after AP induction when compared to young group (aged: 1.8 *vs* young: 0.3, $p = 0.028$) (Fig. 7A and B) Histological changes were not observed in the pancreas in animals of the control and sham groups. Results are in histologic score.

Discussion

Although early reports did not demonstrate differences between young and aged animals with acute pancreatitis (AP) [22], the present study demonstrates an increased severity of AP in aged rats. The main point in the experimental model of the present study is related to the amount of sodium taurocholate injected into the pancreatic duct in young and elderly rats to induce AP. Although old rats have a higher weight compared to younger animals their pancreas size does not significantly increase with age [23] Therefore, the same amount of sodium taurocholate should be injected

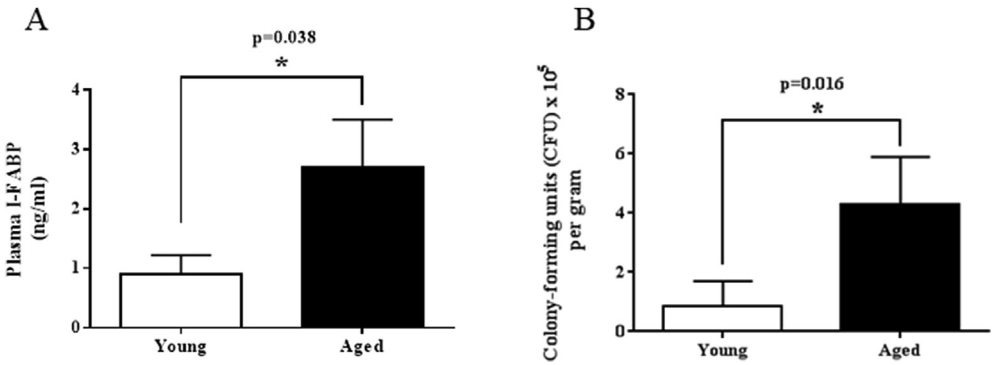

Fig. 5. Effect of aging on ileal fatty acid binding protein (I-FABP) (5A) and on pancreatic infection 5B). Groups of animals Young and Aged were submitted to acute pancreatitis (AP). Data are expressed as mean ± SEM. $^{*}p < 0.05$.

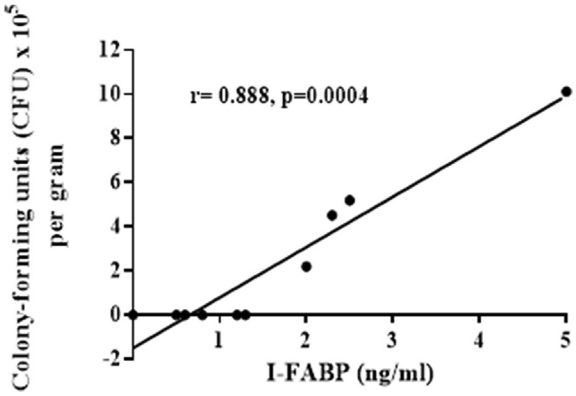

Fig. 6. Correlation between the plasma I-FABP levels and the pancreatic infection. Groups of animals Young and Aged were submitted to acute pancreatitis (AP). Linear regression analysis was performed with use of the Spearman correlation coefficient.

into the pancreatic duct regardless of the rats age. We did not evaluated the fat body composition of our animals however in a recent report [24] evaluating the body composition in young and old rats the following results were obtained:percent body fat in young rats 25,19% in old rats 28,95%. We concluded therefore that this increasing in percentage of body composition in aged animals may not be an important factor in the inflammatory response related to AP in old population.

In the present study higher levels of serum amylase was observed in aged animals when compared to the young ones, corroborating the findings of a previous report using mice and repeated cerulein injections to create a model of AP in ageing animals [6]. Although plasma amylase levels have been considered in animal models, a marker of AP severity [25], in the clinical situation no correlation exists between severity of the disease and amylase plasma levels [26].

Increased TNF-α levels occur at the early phase of the inflammatory process and is followed by IL-6 secretion. On the other hand, IL-6 may have a suppressive effect on TNF-α expression [27] and thus downstream cytokines may be better biomarkers of the AP severity [28]. The finding in the present study of higher levels of TNF-α in ascitic fluid and in plasma in young rats when compared to aged animals submitted to AP do not correspond to the findings of an earlier study in which no differences were found in plasma levels of TNF-α between young and old animals [6]. On the other hand, in our study higher serum and ascitic fluid IL-6 levels were observed in aged animals compared to the young rats with AP. We can thus conclude that TNF-α elevation in AP could not be the direct cause of elevated plasma IL-6 concentrations in aged animals as has been previously reported [6]. We may also conclude that IL-6 is a good biomarker of severity of AP in the elderly population. In fact, higher IL-6 serum levels after AP have been described in aged animals when compared to young ones [6]. Since serum levels of IL-6 are considered a marker of pancreatitis severity [29] it can be stated that severity of AP is greater in elderly animals when compared to young animals. The increase of serum IL-10 in animals of AP aged group when compared to animals of AP young group was not

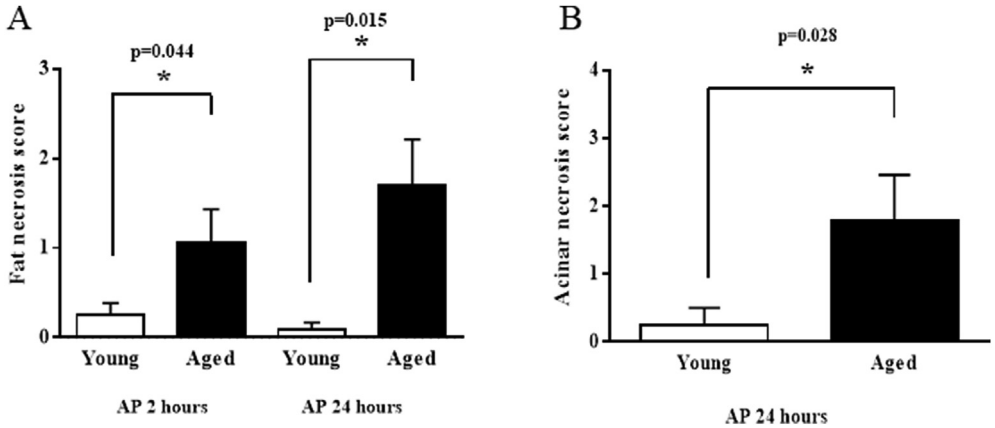

Fig. 7. Effect of aging on histological analysis of the pancreas. Groups of animals Young and Aged were submitted to acute pancreatitis (AP). Fat necrosis (7 A) and acinar pancreatic necrosis (7 B) were significantly increased in the aged group compared to young group. Data are expressed as mean ± SEM * $p < 0.05$.

estatiscally significant (Fig. 1C) what suggests a prevalence of proinflammatory (IL-6) over anti-inflammatory cytokines (IL-10) in aged animals when compared to the AP in young subjects. The finding of an increased severity of local response in older animals in this study is in discordance with a previous study that reported less severe local lesions in older animals and a systemic worse response [5]. This discrepancy is possibly due to methodology differences between the two studies, related to animal type and method of PA induction.

The increased serum levels of ALT and AST in aged animals in this study is related to an increased liver damage compared to the young animals. ALT is elevated in aged animals at 2 h after AP induction and remained elevated 24 h after AP induction.

Liver mitochondrial dysfunction was found not only in AP aged animals but also in sham aged animals. Unexpectedly this pre-existing severe mitochondrial dysfunction remained unchanged after AP induction in aged animals. In the opposite direction, young animals with AP presented less severe mitochondrial dysfunction mainly due to uncoupling of oxidative phosphorylation, which was in accordance with a previous study from our laboratory when it was demonstrated that this dysfunction is always transient [11]. MDA is elevated in AP young group when compared to young sham group, however, as MDA is already increased in aged animals sham group, due to increased mitochondrial production of reactive oxygen species, it does not have further increase after AP induction in these older animals. This mitochondrial dysfunction already present in aged sham animals may be implicate in the decline of mitochondrial oxidative phosphorylation, increasing mitochondrial production of reactive species and increased extent of oxidative damage to DNA, proteins and lips and therefore contributing to hepatic damage after AP insult. Mitochondrial dysfunction related to ageing is already well known [30–33] however, the lack of further worsening of this dysfunction after AP in aged animals is a new observation and needs further investigation.

It is also possible that intestinal mitochondria presents the same dysfunction as demonstrated in liver mitochondria and be at least in part responsible for the increased intestinal barrier dysfunction observed in aged animals with AP. In fact, the inhibition of nicotinamide adenine dinucleotide phosphate oxidase, a source of oxidants in the intestine of rats with AP reduces the intestinal barrier dysfunction in this situation [34]. The present of systemic mitochondrial dysfunction in the older population however needs further investigation.

Multiple organ failure represented by lung damage [35], measured by pulmonary myeloperoxidase (MPO) activity, kidney dysfunction, demonstrated by urea and creatinine increases, and elevation of glucose plasma levels in aged animals at two and 24 h after AP induction when compared to young animals indicate a more severe systemic damage in the aged group. The increased pancreatic damage observed in the aged animals in the present study may also be secondary to this increased systemic inflammation as has pointed out in previous report [6]. Another study however, found that pancreata of older animals had less edema, decreased inflammatory response and increased bacterial infiltration [5]. In that study the increased of pancreatic bacterial infiltration in aging animals was attributed to the decrease of pancreatitis-associated-protein in aging animals [5], however intestinal bacterial translocation was not evaluated in the study. In the present study an increased bacterial translocation was observed with an increased pancreatic infection in aged animals associated to gut barrier failure. The intestinal barrier dysfunction may be evaluated by several methods. In the present study enterocyte damage was assessed by measuring plasma levels of ileal fatty acid binding protein (I-FABP) [36]. Therefore, high plasma levels of I-FABP indicates gut epithelial cells injury during AP and may be related to bacterial translocation marker of this disease [18,19] being an early non-invasive marker of intestinal damage [37,38]. In the present study there was a significant positive correlation between plasma I-FABP levels and pancreatic infection and higher levels of I-FABP in aged rats compared to the young animals, indicating a more severe intestinal damage in this group of elderly animals. Previous clinical study in patients with acute pancreatitis also demonstrated that patients with bacteriemia, infected necrosis and more severe disease had higher I-FABP levels [18]. A previous study from our laboratory also demonstrated an increased intestinal barrier dysfunction in aged animals with AP that may be implicated in the increased systemic inflammation in AP elderly population [7].

Reduction in the volume of the intestinal microvessel network in aged rats [39] increasing the possibility of intestinal ischemic damage may also contribute to intestinal barriers dysfunction observed in AP induction in aged rats [7].

Previous studies have shown that administration of equal amount of bacterial endotoxin induced an increased systemic inflammation in multiple organs in aged mice when compared to young [40–43]. The mechanisms of this increased inflammatory response induced by sepsis or trauma in elderly population have been discussed in a recent review [44]. In a previous study we could demonstrate that the systemic inflammation in AP also includes the small bowel [7] and may induce increased intestinal barrier dysfunction with bacterial and endotoxin translocation that may further contribute to the severity of AP in aged animals.

In conclusion we observed in aged animal with AP not only an increased systemic inflammation and damage but also an increased local lesion. We also observed a significant increased intestinal barrier dysfunction with increased bacterial translocation that may be related to the increased systemic inflammation and damage observed in aged rats with AP. The increased pancreatic infection in aged rats with AP may be associated not only to the increased steatonecrosis and acinar necrosis observed in aged rats but also to the increased bacterial translocation observed in this population These findings may have significant therapeutic implications in the clinical setting.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pan.2019.06.005.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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