Pathology – Research and Practice 210 (2014) 1011–1017

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original Article

A new pathological scoring method for adrenal injury in rats with severe acute pancreatitis

Wenhong Deng[a,1], Yuanjian Hui[b,1], Jia Yu[a], Weixing Wang[a,*], Sheng Xu[c], Chen Chen[a], Xincheng Xiong[a]

[a] Department of General Surgery, Renmin Hospital of Wuhan University, 238 Jiefang Road, 430060, Hubei Province, People's Republic of China
[b] Department of General Surgery, Taihe Hospital of Hubei Medical University, Shiyan 442000, Hubei Province, People's Republic of China
[c] Department of General Surgery, People's Hospital of Guangxi Zhuang Autonomous Region, 530021, Nanning Province, People's Republic of China

ARTICLE INFO

Article history:
Received 7 March 2013
Received in revised form 26 August 2013
Accepted 14 April 2014

Keywords:
Acute pancreatitis
Adrenal glands
Corticosterone level
Pathological Scoring

ABSTRACT

These studies investigated the appearance and function of adrenal glands in rats with severe acute pancreatitis (SAP) and established a new histopathological score to evaluate adrenal histopathological changes. Severe acute pancreatitis relied on retrograde infusion of 5% sodium taurocholate into the bile-pancreatic duct. The damage of SAP was estimated by serum amylase, secretory phospholipase A_2 and pancreatic histopathology. Light and electron microscopy of adrenal gland, and the levels of serum corticosterone were investigated. These results showed that the generally ascending trend of adrenal pathological score was inversely proportional to the generally descending trend of serum corticosterone levels, but parallel with the changes of pancreatic histopathology. Herein, the new adrenal histopathological score was effective in the evaluation of adrenal injury following SAP. It may indirectly reflect the variation of serum cortisol levels and the severity of pancreatitis to a certain extent.

Introduction

Severe acute pancreatitis (SAP) was an acute abdomen-characterized disease with elevated levels of pancreatic enzyme; and was often accompanied by systemic inflammatory response syndrome and remote multiple organ dysfunction [1].

Recently, some studies showed that adrenal insufficiency was of great importance in critical diseases such as shock, trauma, pancreatitis, and systemic inflammatory response [2–5]. A prospective observational multicenter study revealed that adrenal insufficiency was found in 16% of all patients with severe acute pancreatitis; and the mortality rate in patients with adrenal insufficiency was significantly higher than patients without it [6]. Other studies have supported the existence of adrenal insufficiency in the early phase of pancreatitis patients [7,8].

In performing the short corticotrophin stimulation test, a cortisol response of less than 9 μg/dl has been suggested as being a sign of adrenal insufficiency [6,9]. Some iconography results, such as CT or MRI, have been used to define the adrenal injury [10–12]. However, all of these clinical studies lacked a pathological diagnosis, which was the gold standard of adrenal injury. In the animal experimentation, authors referred to adrenal histopathological scores, but the criteria used comprised only necrosis area [13,14]. Herein, we characterize a new pathological standard for adrenal injury in SAP that we believe overcomes the shortcomings of previous pathological scores, aimed at determining the severity of adrenal pathological injury in severe acute pancreatitis.

Materials and methods

Animals

Male SPF wistar rats (200–250 g). were obtained from the Center of Experimental Animals of Hubei Academy of Medical Sciences, Wuhan, China. All experiments were conducted in accordance with the principles of the 1983 Declaration of Helsinki as judged by the ethics committee of Wuhan University. All animals were kept at room temperature with natural day–night light cycles, and free access to water.

Experimental groups

All the rats were randomly assigned into either the sham operation group (CON group, $n = 50$) or the severe acute pancreatitis

* Corresponding author. Tel.: +86 027 88041911 86443; fax: +86 027 88042292.
E-mail address: sate.llite@163.com (W. Wang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.prp.2014.04.009
0344-0338/

group (SAP group, $n = 58$). Rats in the CON group were further subdivided into five subgroups for the time points 1 h, 3 h, 6 h, 12 h, and 24 h ($n = 10$/each time point); rats in the SAP group were also subdivided into five subgroups for the time points 1 h ($n = 10$), 3 h ($n = 10$), 6 h ($n = 10$), 12 h ($n = 12$), and 24 h ($n = 16$). The animals were fasted overnight but given fresh tap water ad libitum. Rats in the SAP group were laparotomized in the midline under anesthesia with intraperitoneal pentobarbital sodium (30 mg/kg).

The pancreatic bile duct was cannulated through the duodenum. Then SAP was induced by a standardized retrograde infusion of a freshly prepared 5% sodium taurocholate solution (1 ml/kg, STC, Sigma, dissolved in saline solution, 0.9%) at a speed of 0.1 ml/min at a steady pressure. The rats in the CON group were given similar volumes of saline solution but not sodium taurocholate solution.

For the two groups, saline solution (20 ml/kg) was injected subcutaneouly into the back to compensate for fluid loss. Rats that survived were sacrificed by taking blood via heart puncture. Blood samples were centrifuged for 15 min at 3000 rpm. Serum was collected and stored at −80 °C until the analysis. After sacrifice, the head of the pancreatic tissue and the left adrenal gland were fixed in 4% PBS-buffered formaldehyde. The right adrenal gland was placed in 3% glutaraldehyde for electron microscopy analysis. The remaining pancreatic tissue was immediately snap frozen in liquid nitrogen and stored at −80 °C for assay.

Serum assay

Serum amylase (AMY) levels were measured by an automatic biochemistry analyzer with standard techniques (Olympus Optical Ltd., Japan).

Serum secretory phospholipase A_2 ($sPLA_2$) activity was measured by sPLA2 Assay Kit according to the manufacturer's instructions (Ann Arbor, MI).

Serum corticosterone levels were measured by a commercially specific Enzyme Linked Immunosor bent Assay kit (Adlitteram Diagnostic Laboratories, USA).

Light microscopy

After being embedded in paraffin and sectioned, the pancreatic tissue and the left adrenal gland were taken for pathological examination with H&E staining. The sections were evaluated by two independent expert pathologists who were blind to the experiment. The changes in the pancreatic histological assessment were scored based on edema, inflammation, hemorrhage and necrosis according to the scale described by Schmidt et al. [15]. The histopathologic evaluation of the left adrenal gland was decided by the standardized scoring method (Table 1). The histopathologic scoring method applied to each of the 20 fields in each individual animal and the average of the scores was used as the final histopathologic score.

Electron microscopy

The right adrenal glands (about 1 mm^3) were fixed in 3.6% glutaraldehyde in 0.1 mol/L cacodylate buffer, and then postfixed in 2% osmium tetroxide. The samples were dehydrated in a graded series of ethanols and propylene oxide. All samples were embedded in Epon 812. Sections with 1 μm were cut from each block on a reichert ultramicrotome. After being stained with lead citrate and uranyl acetate, the sections were observed under tecnai transmission electron microscope.

Table 1
Adrenal histopathologic scoring method.

Cell degeneration (including granular degeneration, adipose degeneration and vacuolar degeneration)	
0	0–1 cell degeneration/HPF
0.5	2–5 cell degeneration/HPF
1	6–10 cell degeneration/HPF
1.5	11–15 cell degeneration/HPF
2	16–20 cell degeneration/HPF
2.5	21–25 cell degeneration/HPF
3	26–30 cell degeneration/HPF
3.5	31–35 cell degeneration/HPF
4	≥36 cell degeneration/HPF
Cell necrosis	
0	Absent
1	Focal occurrence of 1–5 necrotic cells/HPF
2	Diffuse occurrence of 1–5 necrotic cells/HPF
3	Same as 2+ focal occurrence of 1–5 necroticcells/HPF
4	Diffuse occurrence of ≥6 necroticcells/HPF
Enlarged blood sinusoid	
0	Absent
0.5	1–10 enlarged blood sinusoid/HPF
1	11–20 enlarged blood sinusoid/HPF
1.5	21–30 enlarged blood sinusoid/HPF
2	≥31 enlarged blood sinusoid/HPF
Leukocyte or neutrophil infiltrate	
0	Absent
1	1–5 infiltrated cell/HPF
2	≥6 infiltrated cell/HPF

The histopathologic scoring method applied to each of the 20 fields in each individual animal. HPF, high power field (400×).

Statistical analysis

The results were presented as means ± standard deviation values. Ten animals of each subgroups were taken into account in the statistical analysis except for mortality rate. The difference was performed by one-way analysis of variance (ANOVA) with the SPSS statistical package (SPSS 16.0 for Windows; SPSS Inc., Chicago, IL). Linear correlation between two groups was analyzed using spearman correlation test. A value of $P < 0.05$ was regarded as a significant difference.

Results

Mortality rate

All animals survived in the CON subgroups until sacrifice. In the SAP subgroups 12 h ($n = 12$), 2 rats died and the mortality rate was 16.7%. In the SAP subgroups 24 h ($n = 16$), 5 rats died, therefore the mortality rate was 31.3%.

Serum AMY, $sPLA_2$ and corticosterone levels

Compared with the CON group, serum AMY levels were significantly increased gradually at each time point in the SAP subgroups ($P < 0.05$) (Fig. 1A).

Serum $sPLA_2$ levels in the SAP subgroups elevated from 1 h, peaked between 3 h and 6 h, and then declined to a low level, but higher that in the CON group ($P < 0.05$) (Fig. 1B).

Serum corticosterone levels in the SAP subgroups elevated and peaked from 1 h until 3 h, and then declined to a much lower level than in the CON group until 24 h ($P < 0.05$) (Fig. 1C).

Histopathological score

Representative changes in pancreatic tissue are shown in Fig. 2. The pancreatic injury was estimated, including edema,