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Review Article

The Role of IMA in the Prognosis of Acute Pancreatitis and its Correlation with the NF-κB-Mediated Inflammatory Response

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ABSTRACT

The aim of this study was to investigate the correlation between the serum expression of IMA in AP rats and disease severity and to clarify the relationship between the IMA and NF-κB-mediated inflammation. A rat AP model was established and blood samples from each group were analysed at different time points. After the experiment, the pancreatic tissues of the rats were collected for pathological examination and the protein expression of NF-κB and NF-κB p65 in the pancreatic tissue homogenate was assessed. The serum IMA concentration in the SAP group was greater than that in the MAP group. The levels of the NF-κB and NF-κB p65 proteins were increased in the MAP and SAP groups in a time-dependent manner. α-AMY, TNF-α and IL-6 were increased at all-time points in the MAP group and SAP group and were more significantly increased at 24 h in the SAP group. In terms of pathological changes in pancreas, renal and lung tissue lesions, the damage in the SAP group was more obvious than that in the MAP group. IMA is closely related to the severity of AP. NF-κB and its related cytokines were correlated with IMA expression levels.

Keywords: Acute pancreatitis; Inflammatory response; Cytokines; Serum expression

INTRODUCTION

Acute Pancreatitis (AP) is one of the most clinically common acute illnesses and is also a common acute abdominal disorder and its prevalence is increasing. AP has the characteristics of rapid onset, rapid progression, multiple complications and high mortality. Up to 40-70% of AP patients will develop pancreatitis-related infection in the late stage and severe cases can develop sepsis, leading to Multiple Organ Failure (MOF). Early identification of high-risk patients who may develop SAP remains a challenging task [1].

Existing scoring systems, such as the acute pancreatitis Bedside Severity Index (BISAP), can effectively predict the severity and prognosis of AP. Other scoring systems include the Ranson score, the revised Atlanta scale, the Acute Physiological and Chronic Health Assessment (APACHE) II and the Sequential Organ Failure Assessment (SOFA). However, scoring systems tend to be very complex and sometimes rely on multiple surveys, which increases the time and resources required. Different biomarkers can be used to determine the severity of acute pancreatitis, such as C-Reactive Protein (CRP), serum procalcitonin and Interleukin 6 (IL-6), which are the most commonly used biomarkers, but none of these biomarkers can accurately indicate the severity of the disease.

In recent years, Ischaemia-Modified Albumin (IMA) has been recognized as a valuable predictor of disease severity and has been studied and recognized as an inflammatory marker for a variety of acute and chronic diseases. IMAs are a class of nonfunctional serum albumin with a reduced ability to bind to transition metal ions due to tissue ischaemia and are considered a candidate biomarker for acute coronary syndrome.

Early activated inflammatory factors such as IL-1, Tumour Necrosis Factor-α (TNF-α) and inflammatory mediators in acute pancreatitis can cause systemic inflammatory response syndrome and multiple organ failure through a series of cascaded amplification effects. The activation of the transcription factor NF-kB is a key pathway that promotes the transcription of these pro-inflammatory genes. Studies have shown that the IMA is correlated with AP severity, Ranson and BISAP scores and

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procalcitonin levels. This finding is consistent with the previous research results of our team. The plasma IMA level in SAP patients is significantly greater than that in non-SAP patients, which may be related to greater permeability of the pancreatic capillary wall and a rapid decrease in blood flow after injury. However, the role of the IMA in the prognosis of acute pancreatitis and its association with NF-κB-mediated inflammatory responses are unclear [2].

Our study was designed to evaluate the role of the IMA in the prognosis of acute pancreatitis and its association with NF-κB-mediated inflammatory responses and to analyse the significance of the IMA as a predictive marker adjacent to classical biomarkers of inflammation or severity, such as serum amylase (alpha-AMY), TNF-α, IL-6 and IL-8.

LITERATURE REVIEW

Groups

SD male rats aged 6-8 weeks were randomly selected for this study. The rats were subjected to a 12-h light/dark cycle, a temperature of 22 ± 1°C, a relative humidity of 60 ± 5% and standardized feeding. The rats were randomly divided into four groups. The plants were divided into a blank control group (control), a Sham Operation group (SO), a Mild Disease Group (MAP) and a Severe Disease Group (SAP).

Establishment of the animal model

The MAP group and SAP group were subjected to 2.0% and 4.0% retrograde cholangiopancreatinal injection, respectively. In the SO group, an equal volume of normal saline was injected into the pancreatic duct. The blank control group did not receive any treatment.

Treatment of experimental specimens

Following successful preparation of the animal model, blood samples were collected from the rats in the model group at 0, 3, 6, 12 and 24 h after the model was generated for the detection of serum amylase, lipase and inflammatory factors. After the experiment, the morphology of the pancreas and surrounding organs was observed, the pancreas, lung and kidney tissues were cut with sterilized surgical scissors and the kidney and lung tissues were removed, fixed by soaking in 4% paraformaldehyde and sent to the pathology room for preparation of sections. The tail tissue of the pancreas was soaked in dilute formaldehyde (intended to be a paraffin specimen) and other pancreatic tissue was frozen in liquid nitrogen at -70°C (for Western blot detection).

The levels of α-AMY, IMA and inflammatory cytokines were used to detect the expression of αAMY

The serum levels of TNF-α, IL-6 and IL-8 were determined by ELISA. The level of IMA in serum was detected by the ACB assay.

Histopathological examination

Rat pancreatic tissue was fixed with 10% neutral buffered formalin and pancreatic HE stained tissue sections were prepared. According to the Schmidt method, pathological grading was performed and evaluated by two senior pathologists in a double-blinded manner; take the upper lobe of the right lung for routine pathological examination and refer to the Osman lung histological scoring standard for lung injury scoring; take a portion of kidney tissue and fix it with paraformaldehyde. Perform routine embedding, sectioning and HE staining and observe the pathological changes of the kidney under a light microscope. The tissue sample slices were taken using an upright microscope with a field of view of × 200 times [3].

The NF-κB and NF-κB p65 protein levels in pancreatic tissue were detected by Western blotting

An appropriate amount of pancreatic tissue was cut and placed in a centrifuge tube. Then, the lysate was added, the tissue was crushed with ultra-sonication in an ice bath and the supernatant was retained. The protein expression of NF-κB and NF-κB p65 was detected by western blotting, the optical density of each band was determined by image analysis software and the protein expression was calculated.

Correlation between α-AMY and NF-κB p65

Pearson correlation analysis was used to determine whether the αAmy concentration was correlated with the surface level of the NF-κB p65 protein.

Statistical analysis

The statistical data were processed with Graph Pad prism 9.5. Counting data are expressed as a percentage (%) and the *Chi-square* test was used. All measurement data were tested for normality and are expressed as the mean ± SD. For measurement data with homogeneity of variance, the independent sample t test was used for comparisons between two groups. Pearson correlation analysis was used to construct scatter plots to determine the correlation of indicators and test them. $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ indicate statistical significance. All of the data presented in this article were subjected to at least three independent experiments.

DISCUSSION

The experimental animal model was successfully verified

At each time point, α-AMY increased in both the MAP group and the SAP group. Pathology revealed that the gross morphology of the pancreas in the MAP and SAP groups showed obvious oedema, haemorrhage, necrosis and extensive necrosis of the peripancreatic adipose tissue. With the prolongation of time, pancreatic injury was aggravated. At the same time point, the

degree of pancreatic injury and necrosis of SAP was more obvious. These findings indicate successful modelling [4].

Serum IMA expression levels of rats in each group at different time points

Serum IMA was detected by the ALB cobalt binding method. There was no significant difference in the IMA at 24 h between the sham operation group and the control group. The IMA in the MAP group and SAP group increased at each time point and the IMA in the SAP group increased significantly. With increasing time, both the SAP group and MAP group showed an increasing trend.

Expression levels of α-AMY, TNF-α, IL-6 and IL-8 in the serum of each group

The expression levels of α-AMY, TNF-α, IL-6 and IL-8 in the serum samples of each group were detected by ELISA. Compared with those in the control group, α-AMY, TNF-α and IL-6 in the MAP group and SAP group were increased at all-time points and the increases were more significant in the SAP group. Moreover, the α-AMY and TNF-α levels in the SAP group were significantly greater than those in the other time points and in the MAP group at 24 h, but there was no significant difference in the IL-6 group at any time point. IL-8 was elevated in both groups, but there was no significant difference between the two groups.

Expression of NF-κB and NF-κB p65 protein in pancreatic tissue samples from rats in each group

The expression of NF-κB and NF-κB p65 in pancreatic tissue samples from all groups was detected by western blot analysis. Compared with that in the control group, the expression of NF-κB and NF-κB p65 in the MAP group at 24 h was increased and that in the SAP group at 12 h and 24 h was increased. The difference in the expression level between the SAP group and the control group was significant [5].

Correlation analysis of α-AMY and NF-κB p65

Pearson correlation analysis indicated a positive correlation between α-AMY and NF-κB p65 expression.

Comparison of HE staining of pancreatic tissues from each group

According to the results of the quantitative evaluation of the effect of lesion (H and E) staining, histopathological examination of pancreas showed that islet cells in control group and SO group had normal distribution, round nucleus, cytoplasmic distribution, clear structure and no degeneration and necrosis. Compared with the control group, pancreatic cell oedema, inflammatory cell infiltration, bleeding and necrosis were not obvious in MAP 6 h group and SAP 6 h group. MAP 12 h group and SAP 12 h group showed obvious inflammatory manifestations: Widening of interlobular space, interlobular oedema, obvious inflammatory cell infiltration, acinous cell swelling and a small amount of haemorrhage and necrosis. MAP 24 h group and SAP 24 h group showed extensive coagulation necrosis with bleeding, unclear cell structure in the necrotic area and a large number of inflammatory cells infiltrated in parenchyma, among which the SAP 24 h group was the most severely damaged.

Comparison of HE staining of renal tissues from each group

Compared with the renal tissue structure of rats in the control group and sham operation group. In the 6 h group of mild to severe acute pancreatitis, the glomerular structure was still intact, the renal tubules were slightly swollen and the interstitium was congested; two groups of 12 h renal glomeruli showed swelling, structural damage, gradually disordered arrangement, significant swelling of renal tubules, narrowing of renal lumen, congestion of renal interstitium and infiltration of inflammatory cells; two groups of 24 h renal glomerular swelling, capillary network cracks, widespread renal tubular lesions, disordered arrangement, swelling, almost no narrowing of the lumen, epithelial cell necrosis, structural disappearance, severe renal interstitial congestion and significantly increased inflammatory cell infiltration.

Comparison of HE staining of lung tissues from each group

Compared with the control group, pathological changes such as inflammatory cell infiltration, alveolar wall edema and bleeding were observed in the lung tissue of rats in each model group; at the same modeling time, the pathological changes in the severe acute pancreatitis group were more significant than those in the mild acute pancreatitis group. At different times, the pulmonary edema, inflammatory cell infiltration and bleeding in the 12-hour group of mild and severe acute pancreatitis worsened compared to the 6-hour group; the edema, infiltration of inflammatory cells and bleeding of lung tissue in the 24-hour group were more severe than those in the 12-hour group [6].

Osman score results of lung tissue in each group

The Osman score results of each group's lung tissue showed that the scores were highest at 12 hours and 24 hours for severe cases, followed by 24 hours for mild cases and there were statistically significant differences compared to the control group and sham surgery group.

In this study, we further identified the role of the IMA in the occurrence and development of pancreatitis from the perspective of molecular biology through the correlation between the IMA content in the blood and pancreatic tissues of model rats and the expression of the p65 protein, which is related to the NF-κB signalling pathway and pathological type and provided detailed evidence for dynamic prediction and targeted therapy.

Ischaemic processes are thought to contribute to the development of AP and ischaemic markers help diagnose and determine the prognosis of this disease. Ischaemia-Modified Albumin (IMA) is produced by the liver and is associated with

interactions between free radicals produced during ischaemia. Studies suggest that the formation of the IMA is the earliest sign of ischaemia and is related to vascular endothelial disorders in the early stage of the disease. Microthrombosis is associated with pro-inflammatory cytokines, prothrombotic autoantibodies and an impaired endothelial barrier and may lead to local tissue ischaemia, oxidative stress and increased albumin oxidation in SAP patients. Therefore, IMA levels are also elevated in patients with COVID-19, deep vein thrombosis and pulmonary embolism.

Rajinder, et al. reported that the IMA increases the incidence of AP, but its effectiveness in terms of prognosis is limited to exploratory studies. The relationship between the two has not been fully evaluated in clinical trials. Some studies have also reported that the common background of acute and chronic inflammatory states, oxidative stress and reactive oxygen species formation leads to increased serum IMA concentrations in patients with noncardiac diseases (such as sepsis, intussusception ischaemia and rheumatoid arthritis). In our study, we evaluated the value of the IMA as a prognostic marker in patients with AP, determined whether the IMA is effective in assessing disease severity and assessed the association of the IMA with routine clinical inflammatory and prognostic markers [7].

Acute pancreatitis is often accompanied by cytokine changes, especially severe acute pancreatitis, where the activation of NF-κB in the pancreas itself, distant organs, tissues and intracellular cells is significantly enhanced, accompanied by the upregulation of various inflammatory cytokines, which may play an important role in the occurrence and development of SAP. NF-κB exists in the body as a dimer composed of Rel, p65, p50 and other molecules and can regulate the expression of the inflammatory factors TNF-α, IL-1, IL-6, IL-8, etc. and regulate the cytokine-mediated inflammatory response. The dominant dimer in the pancreas is the NF-κB p65/p50 dimer. In the classical inflammatory pathway, the NF-κB dimer travels to the nucleus and binds to a common sequence of transcription factors and target genes. Experimentally induced acute pancreatitis has been reported to lead to the apoptosis of pancreatic acinar cells and increased NF-κB activation. Huang, et al. suggested that NF-κB activation was related to the severity of acute pancreatitis. One study reported that exposure to cyanin led to apoptosis and increased the expression of NF-κB p65 in pancreatic cells. Similarly, the expression level of NF-κB p65 and apoptosis of pancreatic cells were increased in the pancreatic tissues of rats with severe acute pancreatitis [8].

Animal experiments showed that the IMA increased at all-time points in the MAP group and SAP group, which may be related to the high permeability of pancreatic capillary endothelial vessels and the rapid decrease in the effective circulating blood volume after damage, indicating that acute pancreatitis can lead to ischaemic changes. The IMA in the experimental group increased with time, which was considered to be related to the prolonged duration of tissue hypoxia, hypoperfusion, inflammation and oxidative damage. The IMA was more significantly elevated in the SAP group at all-time points, which was associated with more severe acute severe pancreatitis injury. α-AMY, TNF-α and IL-6 play a certain role in determining the severity of pancreatitis. With increasing pancreatitis severity and time, the α-AMY level tended to increase, while the corresponding NF-κB p65 protein level also gradually increased and the two were positively correlated. At the same time, from the results of HE, it can be seen that compared with the control group, there were varying degrees of pathological changes in the pancreatic, renal and lung tissues of rats in each group of mild and severe acute pancreatitis. The main manifestation was that with the prolongation of modeling time, the infiltration of inflammatory cells in each group of mild and severe acute pancreatitis increased and cell necrosis accompanied by bleeding occurred. The pathological changes in the severe group were more significant than those in the mild group. The Osman score results of lung tissue showed that the highest scores were obtained at 12 and 24 hours in severe pancreatitis, followed by 24 hours in mild pancreatitis, indicating the release of inflammatory factors in severe pancreatitis (TNF-α, IL-6 and IL-8) have significant damage to multiple organs in the body, even in mild pancreatitis, they have a certain degree of damage to multiple organs and the damage worsens over time. Since pancreatic tissue sampling was limited to pancreatic tail tissue, the degree of NF-κB activation in cells in other parts of the pancreas cannot be explained. However, further studies on the influence of other organs on ischaemia-sensitive target organs are needed [9].

In our study, the levels of serum amylase, lipase and IMA, as well as TNF-α, IL-6, IL-8, IMA and the NF-κB target gene p65 protein, were highly increased in severe acute pancreatitis patients. The IMA correlates with the severity of AP and IMA levels increase as AP progresses. Therefore, the IMA helps clinicians assess the severity of AP early in the disease course. The expression levels of NF-κB and its target genes and related cytokines were correlated with those of IMA and its target genes in the experimental model group.

In addition to the associations of the ischaemic markers IMA and inflammatory markers with NF-κB p65 expression identified in this study, further research is needed to determine the associations between ischaemic vascular endothelial disorders and the IMA in acute pancreatitis, especially in clinical studies evaluating the role of the IMA in acute pancreatitis severity [10].

CONCLUSION

These results suggest that the serum IMA protein is associated with inflammatory markers and NF-κB p65 in rats with acute pancreatitis. However, further studies are needed to explore the mechanisms underlying this association, particularly the association between IMA and impaired vascular endothelial disorders in acute pancreatitis and the potential role of IMA as a candidate biomarker for acute pancreatitis.

ETHICAL APPROVAL

The study was approved by the insititutional review board of The Fifth Affiliated Hospital of Xinjiang Medical University.

CONSENT FOR PARTICIPATE

Not applicable.

CONSENT FOR PUBLISH

Not applicable.

AUTHORS CONTRIBUTIONS

Cheng Geng and Xinjian Xu conceived and designed the study. Junxiang Zhang and Bolin Zhang performed laboratory experiments. Shixing Wu, Xinxin Tian, Tigu A and Hongde Su analyzed data and interpreted the results. Tigu A created the figures and drafted the manuscript; Junxiang Zhang and Shixing Wu edited and revised manuscript. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analysed during the current study are not publicly available due [Reason why data are not public] but are available from the corresponding author on reasonable request.

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