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Letter

Tetrahedral Framework Nucleic Acids Can Alleviate Taurocholate-Induced Severe Acute Pancreatitis and Its Subsequent Multiorgan Injury in Mice

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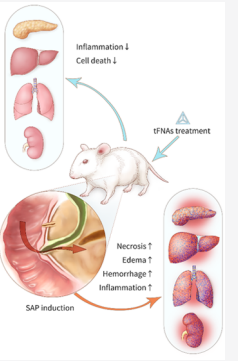
ABSTRACT: Severe acute pancreatitis (SAP) is an inflammatory disease of the pancreas accompanied by tissue injury and necrosis. It not only affects the pancreas but also triggers a systemic inflammatory response that leads to multiorgan failure or even death. Moreover, there is no effective treatment currently that can reverse the disease progression. In this study, tetrahedral framework nucleic acids (tFNAs) were utilized to treat SAP in mice for the first time and proved to be effective in suppressing inflammation and preventing pathological cell death. Serum levels of pancreatitis-related biomarkers witnessed significant changes after tFNAs treatment. Reduction in the expression of certain cytokines involved in local and systemic inflammatory response were observed, together with alteration in proteins related to cell death and apoptosis. Collectively, our results demonstrate that tFNAs could both alleviate SAP and its subsequent multiorgan injury in mice, thus offering a novel and effective option to deal with SAP in the future.



KEYWORDS: *Severe acute pancreatitis, Tetrahedral framework nucleic acids, Multiorgan injury, Apoptosis, Inflammation*

Acute pancreatitis is one of the most commonly seen inflammatory diseases, with a fatality rate of more than 5% among identified patients.[1] Around 20% of AP progressed into severe acute pancreatitis (SAP) within a short period of time, accompanied by pancreatic tissue injury and necrosis.[2] What is worse, SAP eventually leads to multiorgan failure in the human body, mainly affecting the lungs, liver and kidneys.[3] Multiple studies have proved that the dysfunction of the above organs accounts for a considerable amount of death caused by SAP and its complications.[4,5] The commencement of SAP is generally considered to be neutrophil gathering and relocating, followed by leukocyte recruitment and unleashing of cytokines in both tissue and blood.[6] These alterations quickly lead to serious pancreatic inflammation, followed by tissue damage and even necrosis.[7] Besides this, blood cytokines affect multiple organs other than the pancreas via thehcirculation system, causing a series of chemical and biochemical reactions that eventually result in systemic inflammation and multiorgan injury.[6,8,9] Clinically, patients with SAP were given anti-inflammatory therapy on a regular basis; if the situation worsens, patients need to be hospitalized in an intensive care unit and are given vital life support. At present, there is no effective treatment for patients with severe acute pancreatitis, since the underlying mechanism is still misty,[10−12] posing great challenge to clinicians and scientists.

Tetrahedral framework nucleic acids (tFNAs) represent a new kind of nanoparticles that have recently emerged in the scientific realm. tFNAs are self-assembled three-dimensional (3D) structures formed by four single-stranded DNAs (ssDNA).[13] Recent accumulating evidence has indicated that tFNAs possessed a strong capacity in regulating cellular biological behaviors including proliferation, migration, apoptosis, and autophagy. tFNAs were also proved to possess extraordinary anti-inflammatory and antiapoptotic capability in former studies and were utilized in animal models to treat a wide variety of diseases.[14−16] According to our previous studies, tFNAs were used to tackle acute kidney injury (AKI) and showed remarkable capacity in rescuing kidney tissue, according to the significant changes observed in various parameters of renal function.[17] Mi Zhou and others in 2020 also proved that tFNAs treatment could alleviate inflammation both in vitro and in model animals with periodontitis, a well-recognized leading cause for tooth loss worldwide.[18]

Given the fact that tFNAs can exert a strong anti-inflammatory as well as an antiapoptotic effect in different disease settings, we decided to study the influence of tFNAs treatment on severe acute pancreatitis. Here we demonstrate the potential of tFNAs in the disease management of SAP and its subsequent multiorgan injury in a mice model. We have

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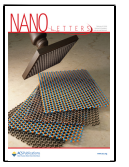

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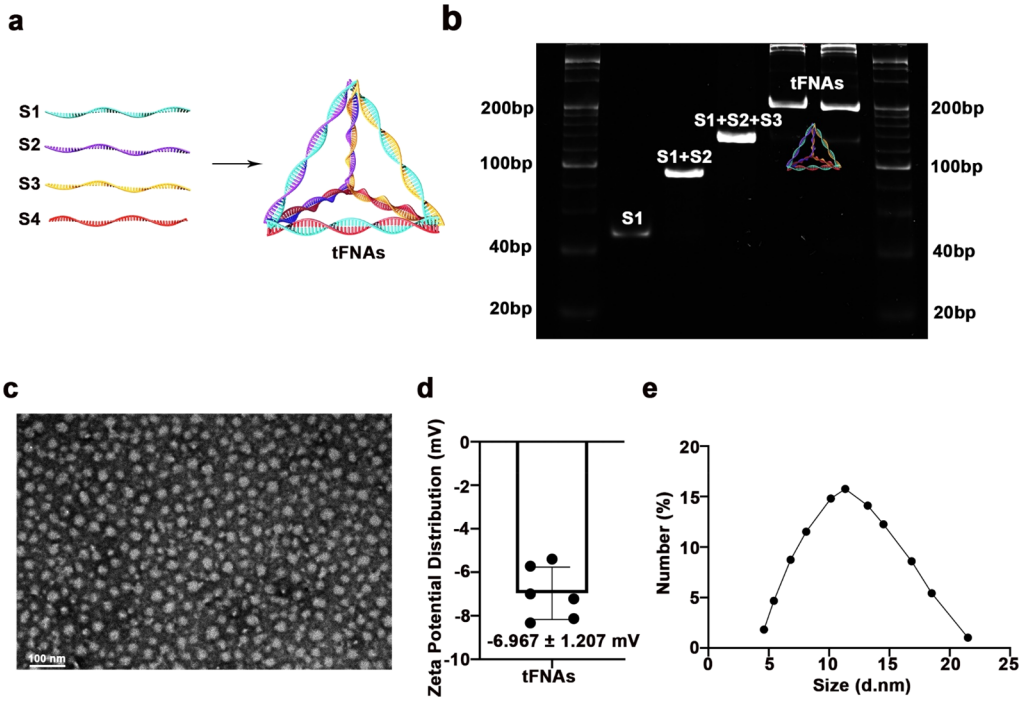

Figure 1. Synthesis and characterization of tFNAs. (a) Schematic illustration of tFNAs. (b) Molecular weights of each ssDNA and tFNAs were detected by 8% PAGE. (c) Synthesized tFNAs detected by TEM. Scale bar: 100 nm. (d) ζ-Potential of tFNAs. (e) Size of tFNAs analyzed by Zetasizer Nano ZS90.

found that tFNAs can alleviate SAP and its subsequent multiorgan injury by specifically suppressing the secretion of inflammatory cytokines both in tissue and blood, meanwhile regulating the expression of specific apoptotic and anti-apoptotic proteins. Besides, tFNAs could also protect multiple organs, including pancreas, lung, liver, and kidney and preserve their normal tissue structures by inhibiting by lymphocytic infiltration a typical inflammatory manifestation.[19]

In retrospect, our results have brought up a novel method of treating SAP as well as its subsequent multiorgan injury and preliminarily illustrated the feasibility and effectiveness of this treatment.

Synthesis and Characterization of tFNAs. The diagram in Figure 1a shows the synthesis of tFNAs. Four single-strand DNAs of equal amount self-assembled into a tetrahedron by complementary base pairing principle (Table S1). Here an 8% polyacrylamide gel electrophoresis (PAGE) was used to illustrate that tFNAs were successfully synthesized (Figure 1b). The molecular mass of tFNAs was shown to be around 200 base pairs (bp) using marker as a reference, consistent with our former findings.[16,20−23] TEM was employed to demonstrate the shape of tFNAs, resembling a triangle on a two-dimensional scale (Figure 1c). The ζ-potential result of tFNAs showed a negative surface charge of 6.967 ± 1.207 mV (Figure 1d), and dynamic light scattering showed that the average size of tFNAs was approximately 10 nm (Figure 1e).

Each side of tFNAs containing 21 bp are the most widely applied tFNAs,[24] and the tFNAs are adopted in our study. According to the previous studies, the biological behaviors of differently sized tFNAs were significantly different.[25−28] Recently, the ssDNA of tFNAs containing different base pairs were analyzed. Each side tFNAs with 7, 13, 17, 21, 26, and 37 bp were the six groups in the study. Although all sized tFNAs could enter cells, tFNAs containing 21 bp on each side had the best membrane-penetrating ability. The proliferation and migration of cells were enhanced, especially with tFNAs containing 21 bp. The study will be helpful to select the optimal size of tFNAs for biological applications.

tFNAs Treatment Alleviated Severe Acute Pancreatitis in Mice. Three experimental groups were created with twenty-one mice randomly assigned to the SAP + tFNAs group (tFNAs group), SAP + saline (Saline) group, and Sham Operation (SO) group. In the tFNAs treatment group, mice were given two separate intravenous injections of tFNAs (125 nM, 100 μL) via tail, 30 and 60 min after SAP induction. For mice in the Saline group, tFNAs solution was substituted with the same volume of saline, while mice in the SO group underwent sham operation. 24 h after the surgery, all mice in three groups were sacrificed (Figure 2a). Fatality rate is one of the most often used indicator to evaluate the effectiveness of drug/medical therapy in treating SAP.[29] Half of the mice in the diseased group died within 24 h after the biliary infusion of sodium taurocholate. On the contrary, mice that received tFNAs treatment after SAP induction achieved 100% 24 h survival rate in our experiment (Figure 2b). In our study, a total number of six biochemical parameters, including amylase, lipase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (CREA), and blood urea nitrogen (BUN) were employed and analyzed to evaluate the extent to which tFNAs treatment can alleviate SAP.[30,31] Amylase and lipase are two indicators for the assessment of treatment efficacy on pancreas.[32] Mice in the saline group manifested a sheer increase in both blood amylase and lipase levels compared to the SO group (Figure 2c,d). In contrast, mice in the tFNAs treatment group managed to maintain relatively low serum amylase and lipase levels, slightly higher than those in the SO group yet significantly lower than those in the SAP group (Figure 2c,d). Serum ALT and AST levels also demonstrated a similar tendency, compared to what we had observed earlier in serum amylase and lipase (Figure 2e,f).

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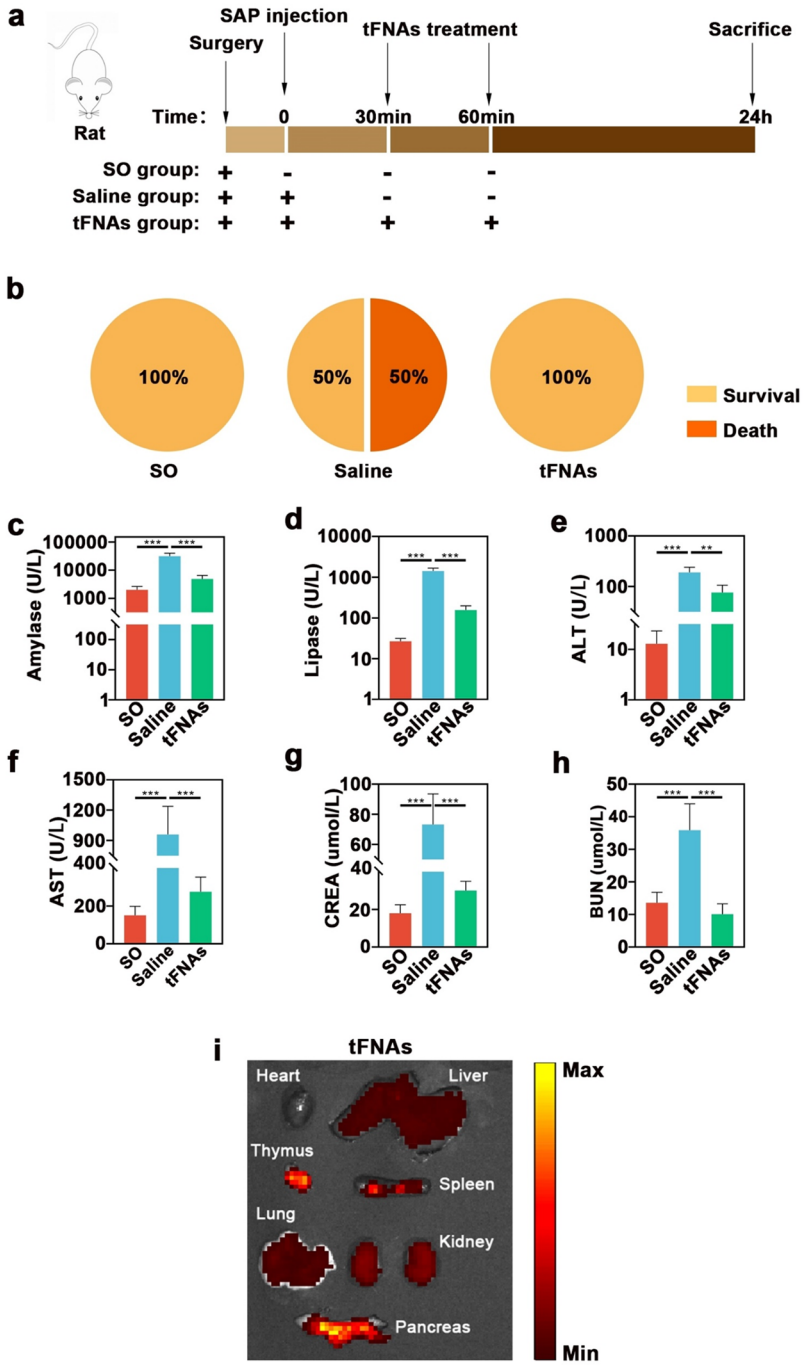

Figure 2. tFNAs treatment alleviated Severe acute pancreatitis. (a) Experimental scheme. (b) Fatality rate of the normal mice and the SAP mice treated with saline and tFNAs. (c–h) Serum amylase, lipase, ALT, AST, CREA,and BUN levels at the end point of the experiment. (i) Fluorescence images of tFNAs biodistribution in the major organs of SAP mice. Throughout, $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. All data were analyzed with one-way ANOVA. The SO group and the tFNAs group contained 10 mice, and the Saline group contained 5 mice. SAP, severe acute pancreatitis; SO, sham operation.

CREA and BUN levels in mice of tFNAs treatment group managed to remain at a normal range while mice in the saline group witnessed great increase in these two indicators (Figure 2g,h). These results altogether supported our previous assumption that tFNAs treatment could ameliorate SAP to a certain degree. *In vivo* bioluminescence imaging was used to illustrate tFNAs distribution within the organism. It was apparent that tFNAs mostly accumulated in major organs including pancreas, thymus, spleen, liver, and kidney (Figure 2i).

tFNAs Treatment Preserved Pancreatic Cells and Inhibit Inflammatory Response in SAP Mice. First and foremost, tFNAs treatment showed considerable potential in rescuing pancreatic cells from pathological death. H&E staining results revealed direction information about visible alterations in pancreatic tissue structure and cellular morphology (Figure 3a). Hemorrhage was one of the most apparent alterations in pancreas of SAP mice, accompanied by destruction of normal island-shape structures and massive lymphocytic infiltration.[6] In particular, pancreatic acinar cells

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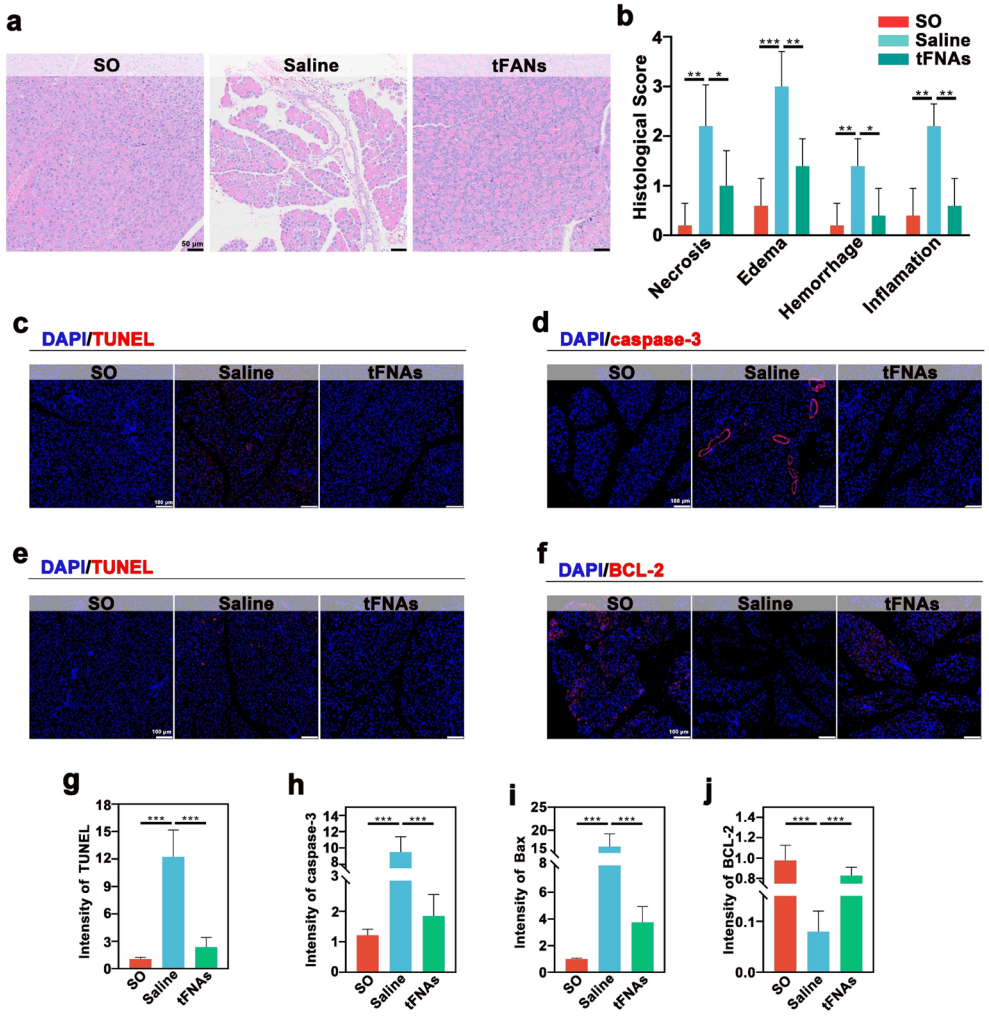

Figure 3. tFNAs treatment preserved pancreatic cells in SAP mice. (a) Representative H&E staining of pancreatic sections at the end point of the experiment. (b) Histologic quantification of acinar cell necrosis, edema, hemorrhage, and inflammation in pancreatic islets at the end point of the experiment. (c–f) Representative immunofluorescence images with the insets showing TUNEL, caspase-3, Bax, and BCL-2-positive (red) at the end point of the experiment (scale bar, 100 μm). (g–j) Quantification of representative immunofluorescence images in parts c–f. Throughout, $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. All data were analyzed with one-way ANOVA. The SO group and the tFNAs group contained 10 mice, and the Saline group contained 5 mice. SAP, severe acute pancreatitis; SO, sham operation.

were mostly distorted or broken with incomplete cell membrane and deep-stained nuclei (Figure 3b). However, in the tFNAs treatment group, pancreatic tissue structure was well preserved, with little hemorrhage seen in the scope and only few scattered lymphocytes, resembling the situation in the SO group (Figure 3b). Results from terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) assay as well demonstrated the effectiveness of tFNAs treatment in the SAP disease model, with obvious decrease in the number of cells that were stained positive (Figure 3c,g). The corresponding immunofluorescence results agreed with what we had found earlier, showing that tFNAs treatment successfully suppressed the expression of caspase-3 and Bax in mouse pancreas, two markers that are believed to be positively correlated with cell apoptosis (Figure 3d,e,h,i). Meanwhile, the expression of BCL-2, which is widely acknowledged as an important kind of antiapoptotic protein, was up-regulated in the pancreatic tissue of mice from the tFNAs treatment group (Figure 3f,j). All these findings pointed to the conclusion that tFNAs could help prevent cell apoptosis in mice throughout the 24 h follow-up period after SAP induction.

tFNAs were proved to effectively regulate the process of cell death and apoptosis. To be more specific, fewer cells were stained positive for TUNEL in the tFNAs treatment group compared to the Saline group. To further explain this phenomenon, multiple specific protein markers were analyzed, including caspase-3, BCL-2, and Bax. The expression of Bax and caspase-3 were proven to be down-regulated, as their staining strength showed a significant decrease in the tFNAs treatment group. These two markers are believed to be positively correlated with the occurrence of cell death and apoptosis. BCL-2, on the other hand, demonstrated remarkable increase in its expression in pancreas, indicating certain antiapoptotic pathway was activated after tFNAs treatment. It is universally supported that serious inflammation and drastic cell death are two crucial factors that lead to unwanted consequences in SAP.[33]

At the same time, tFNAs were proved to possess outstanding anti-inflammatory property on pancreas. Neutrophil infiltration plays a pioneering role and is often regarded as an important driving factor in almost all kinds of acute inflammatory diseases, including SAP.[34−36] TNF-α, which is universally

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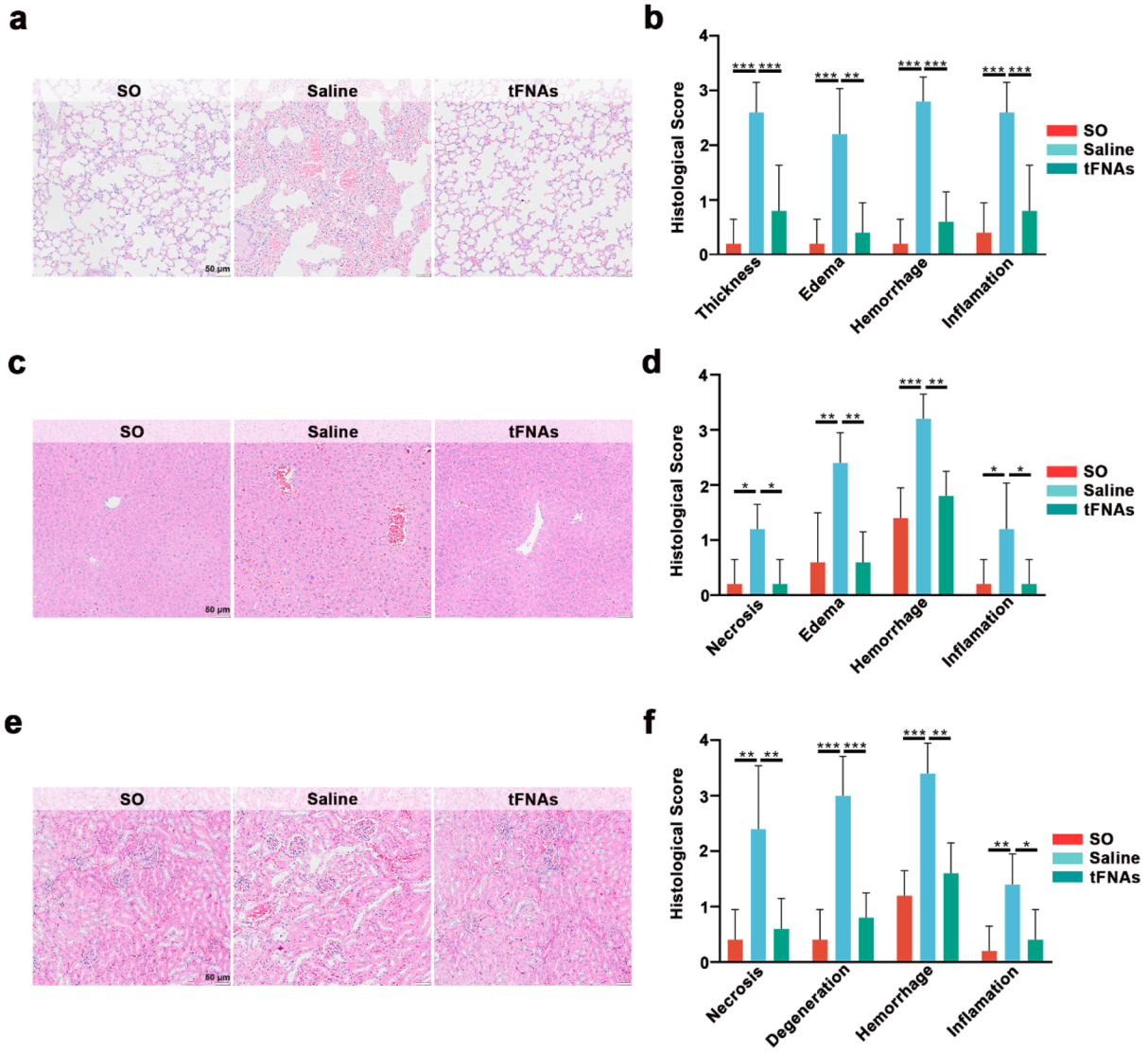

Figure 4. tFNAs treatment alleviated SAP-induced multiorgan injury. (a) Representative H&E staining of lung sections at the end point of the experiment. (b) Histologic quantification of alveolar wall thickness, edema, hemorrhage, and inflammation in lung at the end point of the experiment. (c) Representative H&E staining of liver sections at the end point of the experiment. (d) Histologic quantification of hepatic necrosis, edema, hemorrhage, and inflammation in liver at the end point of the experiment. (e) Representative H&E staining of kidney sections at the end point of the experiment. (f) Histologic quantification of renal necrosis, edema, hemorrhage, and inflammation in kidney at the end point of the experiment. Throughout, $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. All data were analyzed with one-way ANOVA. The SO group and the tFNAs group contained 10 mice, and the Saline group contained 5 mice. SAP, severe acute pancreatitis; SO, sham operation.

regarded as an important cytokine that triggers extensive inflammatory cascades, increased greatly in the saline group but remained at a comparatively lower level in the tFNAs treatment group (Figure S1, Figure S2a,e). Besides, IL-1β and IL-6 expression witnessed significant changes among the three experiment groups. In particular, expression of these pro-inflammatory cytokines were enhanced in the Saline group while in the tFNAs treatment group, fluorescence intensity of IL-1β and IL-6 were significantly weaker (Figure S2b,c,f,g). Thus, myeloperoxidase (MPO), an enzyme that is positively correlated with neutrophil activity, was used to evaluate the degree of neutrophil infiltration in pancreatic tissue of each mouse.[37] As shown in Figure S2d,h, the accumulation of MPO in the saline group was more apparent when compared to the SO group, while tFNAs reduced the fluorescence intensity of MPO markedly in the treatment group.

tFNAs exerted intensive anti-inflammatory effects on mouse pancreas. The expression of many specific proteins associated with inflammation was down-regulated among mice in the tFNAs treatment group compared to those in the Saline group, thus preventing their sequential inflammatory influence on multiple organs within the organism.[38,39]

tFNAs Treatment Alleviated SAP-Induced Multiorgan Injury. To further explore the protective effect of tFNAs treatment on organs other than the pancreas, lung, liver, and kidney were harvested from all animals participating in the experiment, and hematoxylin and eosin (H&E) staining was performed for each obtained organ, respectively. Several biomarkers related to cell death and inflammation were investigated by immunofluorescence staining.

H&E staining of lung showed marked difference between the SAP group and the tFNAs treatment group (Figure 4a,b). Pulmonary alveoli were distorted due to swollen alveolar wall,

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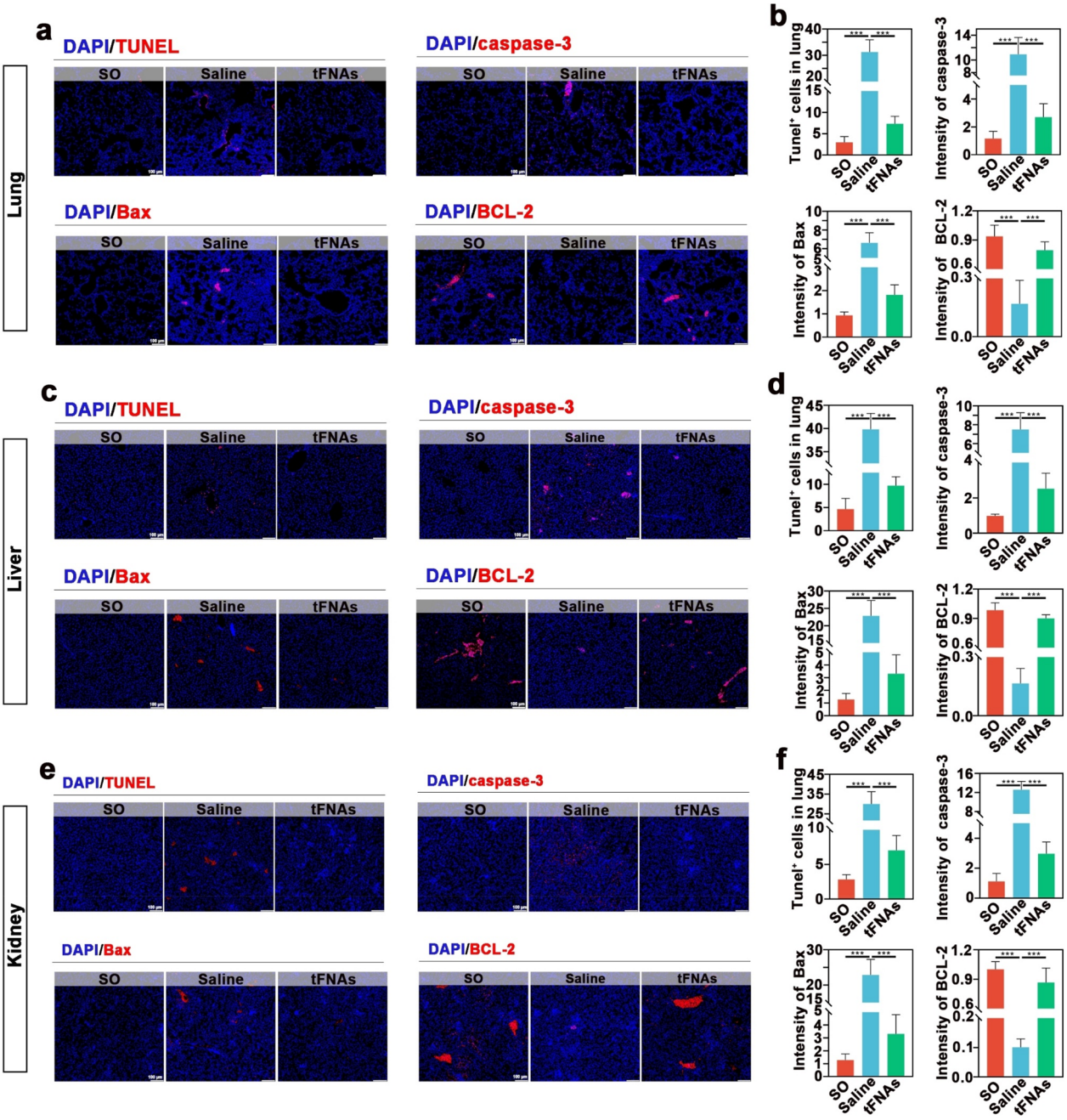

Figure 5. tFNAs treatment preserved cells of multiple organs in SAP mice. (a) Representative immunofluorescence images of lung showing TUNEL, caspase-3, Bax, and BCL-2 -positive (red) at the end point of the experiment (scale bar, 100 μm). (b) Quantification of representative immunofluorescence images in part a. (c) Representative immunofluorescence images of liver showing TUNEL, caspase-3, Bax, and BCL-2 -positive (red) at the end point of the experiment (scale bar, 100 μm). (d) Quantification of representative immunofluorescence images in part c. (e) Representative immunofluorescence images of kidney showing TUNEL, caspase-3, Bax, and BCL-2 -positive (red) at the end point of the experiment (scale bar, 100 μm). (f) Quantification of representative immunofluorescence images in part c. Throughout, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. All data were analyzed with one-way ANOVA. The SO group and the tFNAs group contained 10 mice, and the Saline group contained 5 mice. SAP, severe acute pancreatitis; SO, sham operation.

accompanied by massive lymphocytic infiltration in the interstitial tissue (Figure 4a,b). Similarly, H&E slides for liver also demonstrated apparent changes between the tFNAs treatment group and the saline group (Figure 4c,d). Hepatic sinus is a signature histological structure for normal liver with regularly lined hepatic cell cords; however, in the SAP group, sinus structure was rarely seen while hemorrhage was often spotted in the sinus cavity that is supposed to be clear and vacant (Figure 4c,d). Hepatic cells lost their regular pattern of organization and vacuolar degeneration was the most apparent morphological change seen in our scope (Figure 4c,d).

H&E staining was as well performed on kidney tissue obtained from each participating mouse. In the Saline group, normal structures of renal glomeruli were damaged, as necrosis of renal tubular epithelial cells were commonly seen with broken cell membrane and nuclei (Figure 4e,f). Hemorrhage was another typical histologic feature in the diseased tissue. Both within and between renal glomeruli, clusters of red blood

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cells were discovered (Figure 4e,f). Nonetheless, kidney tissue damage was mitigated among mice in the tFNAs treatment group, as an obvious decrease in epithelial cell necrosis and hemorrhage was easily detected (Figure 4e, f). A pathological scoring system was then employed to evaluate and compare the severity of tissue injury, and our conclusion was that tFNAs preserved mouse kidneys from several aspects, including the reduction in hemorrhage, inflammation, tissue degeneration, and cell necrosis (Figure 4e,f).

Apart from permanent pancreatic injury, SAP also caused trouble by attacking other organs that are closely related to the pancreas.[40] Lung, liver, and kidney are especially vulnerable in SAP disease progression.[41,42] As is reported in the literature, about 20% of patients diagnosed with SAP develop organ injury in the course of the disease, among which about 30% even lost their life due to rampant multiorgan failure.[43] Therefore, nonspecific drugs with a broad therapeutic spectrum are called for in treating SAP as well as controlling the subsequent complications that affect the whole organism and bring severe consequences.[44]

Cell death was evaluated by immunofluorescence stained with TUNEL in lung, liver, and kidney of all experimental animals 24 h after SAP induction. Compared to the SO group, the proportion of dead cells was significantly higher in all three organs in the SAP group with a relatively strong TUNEL stain (Figure 5a−f). However, in the SO group and tFNAs treatment group, tissue cells in the lung, liver, and kidney that stained positive for TUNEL were hardly seen, indicating the fluorescence intensity was reduced by tFNAs treatment (Figure 5a−f). We also counted the number of cells that were stained positive for TUNEL under a random scope and discovered that the number of TUNEL-positive cells in the SAP group was significantly greater than that in the SO group in all three organs, respectively ($P < 0.05$) (Figure 5a−f).

Proteins relevant to cell death and apoptosis were then explored to reconfirm our assumption. Bax, BCL-2, and caspase-3 expression levels were all assessed by immunofluorescence staining (Figure S3a−f). Fluorescence of caspase-3 and Bax were found to be scattered with high intensity in the Saline group; nevertheless, tFNAs treatment imposed a suppressive influence on the expression of these proteins, illustrating a protective effect on tissue and cells in different organs that may suffer from SAP induced dysfunction or even damage (Figure S3a−f).

We as well found that the expression levels of IL-1β, IL-6, and TNF-α increased significantly in lung, liver, and kidney in the saline group, as their fluorescence was quite strong and apparent, while the corresponding specimens in the tFNAs treatment group showed a comparatively faint stain regarding the expression of these three cytokines (Figure S3a−f).

Our results proved the anti-inflammatory and antiapoptotic capability of tFNAs as well as its efficacy in the treatment of severe acute pancreatitis. In recent years, a wide variety of materials were used in treating Severe Acute Pancreatitis; nevertheless, most of them bear apparent shortcomings. A great number of therapies could only exert an apparent protective effect on the pancreas at a dosage that is impractical for clinical use.[45] Some therapies require rigorous patient compliance while some bring serious adverse side effects that harm patients in other aspects.[46,47] Our study, therefore, brings about an unprecedented kind of drug therapy for SAP, which, without prompt and appropriate treatment, could be lethal. What is more surprising is that tFNAs not only alleviate SAP but also impose a positive impact on multiple organs, including lung, liver, and kidney that are quite prone to SAP-induced systemic inflammation and tissue injury. The extraordinary performance of tFNAs has proved its great potential as one kind of nanomaterial with great biosafety and biocompatibility, providing unlimited possibilities for treating SAP and its subsequent multiorgan injury in the near future.

Previous studies have already proved that tFNAs possessed outstanding antiapoptotic and anti-inflammatory properties in a wide variety of diseases. In 2020, tFNAs were proved to prevent osteonecrosis and protect osteoclast from apoptosis.[14,48−50] In 2019, relevant studies showed that tFNAs were effective in maintaining a significantly higher viability of HK-2 cells compared to the control group, thus showing great potential in treating acute kidney injury.[51,52] Besides this, in 2019, Qin and others have also showed that tFNAs treatment helped reduce TBHP-induced RGC-5 cell apoptosis.[53] Xiaoru Shao also demonstrated in her study that tFNAs could prevent PC12 cell apoptosis to facilitate nerve regeneration and shed light on treatment for Alzheimer's disease.[54] The underlying mechanism was quite clear, as these studies mentioned above all looked into the expression levels of Bax, BCL-2, and caspase-3, three proteins that are closely related to the cell apoptotic process. Significant changes in all three markers were found between the disease model group and the tFNAs treatment group, indicating that tFNAs mainly affect cell death via regulating classic apoptotic pathways.

tFNAs were as well shown to inhibit the secretion of various interleukin cytokines that are related to inflammation and cell apoptosis. In 2019, Sirong Shi pointed in a study that tFNAs treatment can down-regulate the secretion of IL-1β. Mi Zhou and others have also pointed out that tFNAs imposed apparent anti-inflammatory effect in periodontitis, since the secretion of TNF-a, IL-1β H&E staining and IL-6 were altered after tFNAs treatment.[14,48,55] All these results were reconfirmed by qPCR and Western Blotting techniques simultaneously, showing a stable and consistent performance of tFNAs.

In conclusion, our study has demonstrated that tFNAs treatment is effective for alleviating SAP and its subsequent multiorgan injury in mice for the first time. tFNAs treatment to a large extent prohibited local and systemic inflammation and cell death to cease the disease progression and help maintain the normal tissue structure of the organs affected by monitoring the secretion of inflammatory factors as well as proteins related to pathologic cell death and apoptosis. tFNAs treatment not only protects the pancreas from acute organ failure, but also alleviates subsequent multiorgan injury that is believed to be a major complication of SAP. Our study might impose a profound and lasting impact on future scientific research that attempts to utilize tFNAs as a treatment option for patients suffering from severe acute pancreatitis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.nanolett.1c05003.

Materials and methods; Table S1, base sequences of the ssDNAs used to construct the tFNAs; Figure S1, blood cytokine concentration; Figure S2, representative immunofluorescence images of the pancreas; and Figure S3, representative immunofluorescence images of multiple organs (PDF)

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Author Contributions

Y. Wang and Y. Li conceived this project. Y. Wang and Y. Li designed the project and collected the data. Y. Wang and S. Gao analyzed the data and wrote the manuscript. S. Gao and Y. Xi provided help during data collection. Y. Lin and Y. Chen provided writing assistance and helped during proofreading of the article.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

SAP	severe acute pancreatitis
tFNAs	tetrahedral framework nucleic acids
AKI	acute kidney injury;
3D	three-dimensional
ssDNA	single-stranded DNAs
PAGE	polyacrylamide gel electrophoresis
AST	aspartate aminotransferase
ALT	alanine aminotransferase
CREA	creatinine
BUN	blood urea nitrogen
TUNEL	terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling
H&E	hematoxylin and eosin.

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