May | 2016

LS045 STUDY REPORT

Efficacy of AN1284 and AN1297 in a Mouse Cerulein-induced Acute Pancreatitis Model

STUDY DIRECTOR
Iris Maimon, Ph.D.
SIGNATURE

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1 INTRODUCTION

Severe acute pancreatitis (AP) is responsible for significant human morbidity and mortality worldwide. Currently, no specific treatments for AP exist, primarily due to the lack of a mechanistic understanding of sterile inflammation and the resultant multisystem organ dysfunction, the pathologic response of AP linked to early death. Various pathological pathways such as inflammation, programmed cell death, necrosis, and oxidative stress are involved in the development and progression of the disease with the end-product of permanent alterations in the morphology and structure of the pancreas.

A mouse model of cerulein-induced acute pancreatitis was utilized in this discovery-level study to evaluate the therapeutic effect of AN1297 and AN1284, using well established and reproducible biomarkers of pancreatitis and inflammation.

1.1 ***Study Number*** LS046

1.2 ***Sponsor*** Professor Marta Weinstock-Rosin, Institute for Drug Research, The Hebrew University of Jerusalem, Israel. Tel: 972-2-6758731, 972-52-5836442; martar@ekmd.huji.ac.il.

1.3 ***Study Director*** Iris Maimon Ph.D, Director of Pharmacology, Luria Scientific Industries, a Division of Salzman Capital Ventures, Ltd. PO Box 587, Herzliya 46105, Israel.

1.4 ***Objectives*** To evaluate the therapeutic effect of AN1284 and AN1297 in a cerulein-induced acute pancreatitis mouse model.

1.5 ***Testing Facility*** The laboratory of Professor Salvatore Cuzzocrea at the University of Messina, located at Via San Filippo Bianchi 19, 98123 Messina

1.6 ***Quality Standard (Is the study in accordance with any internal facility quality standards?)*** These studies were non-GLP.

1.7 ***Study Monitor*** Professor Salvatore Cuzzocrea, University of Messina, located at Via San Filippo Bianchi 19, 98123 Messina

1.8 ***Personnel*** Laboratory staff of Professor Salvatore Cuzzocrea at the University of Messina.

1.9 ***Contact Person*** Dr. Iris Maimon, Luria Scientific Industries, PO Box 587, Herzliya 46105, Israel. Email address: maimon@scvltd.net , Telephone number:+972 542142447.

1.10 ***Study contributors for citation*** a) Andrew L. Salzman, MD, Salzman Capital Ventures, Ltd., Israel; b) Salvatore Cuzzocrea, PhD, Institute of Pharmacology, School of Medicine, University of Messina, Italy.

1.11 ***Conflict of Interest Statement*** The contributors to this study have no conflict of interests.

2 TEST SYSTEM

2.1 ***Species & Gender*** Mice, Male
2.2 ***Strain*** BALB/C
2.3 ***Source*** Harlan Nossan, Milano, Italy
2.4 ***Quarantine*** Animals were quarantined for two weeks before use
2.5 ***Housing*** 6 Animals per cage
2.6 ***Age*** Adult
2.7 ***Body Weight*** 22-25 g
2.8 ***Number of Animals*** 82
2.9 ***Diet and drinking Water*** Standard diet and water ad libitum
2.10 ***Number and Date of OLAW (Office of Laboratory Animal Welfare) Assurance*** A7433-45**,** 07/22/2015.

3 STUDY DESIGN

3.1 *Induction of acute pancreatitis*

This study was carried out in two consecutive experiments, as follows:

Experiment I: Mice were randomly allocated into four groups: (1) sham + saline group: Sham animals, in which saline was administered instead of cerulein (*n*=10); (2) cerulein + vehicle group: mice were treated hourly (×4) with cerulein (50 µg/kg, suspended in saline solution, intraperitoneally [IP], 0.2 ml) and received saline vehicle (0.2 ml, subcutaneously [SC]) 30 min post the first cerulein injection (*n* = 10); (3) cerulein + AN1297 group: mice were treated hourly (×4) with cerulein (50 µg/kg, in saline, IP, 0.2 ml) and received AN1297 (1 mg/kg, SC, 0.2ml) 30 min post the first cerulein administration (*n* = 10); (4) cerulein + AN1284 group: mice were treated hourly (×4) with cerulein (50 µg/kg, in saline, IP, 0.2 ml) and received AN1284 (1 mg/kg, SC, 0.2ml) 30 min post the first cerulein administration (*n* = 10);

Experiment II: Mice were randomly allocated into seven groups: (1) sham + saline group: Sham animals, in which saline was administered instead of cerulein (*n*=6); (2) cerulein + vehicle group: mice were treated hourly (×4) with cerulein (50 µg/kg, suspended in saline solution, intraperitoneally [IP], 0.2 ml) and received saline vehicle (0.2 ml, subcutaneously [SC]) 30 min post the first cerulein injection (*n* = 6); (3) cerulein + AN1297 0.5 mpk group: mice were treated hourly (×4) with cerulein (50 µg/kg, in saline, IP, 0.2 ml) and received AN1297 (0.5 mg/kg, SC, 0.2ml) 30 min post the first cerulein administration (*n* = 6); (4) cerulein + AN1297 2 mpk group: mice were treated hourly (×4) with cerulein (50 µg/kg, in saline, IP, 0.2 ml) and received AN1297 (2 mg/kg, SC, 0.2ml) 30 min post the first cerulein administration (*n* = 6); (5) cerulein + AN1284 0.25 mpk group: mice were treated hourly (×4) with cerulein (50 µg/kg, in saline, IP, 0.2 ml) and received AN1284 (0.25 mg/kg, SC, 0.2ml) 30 min post the first cerulein administration (*n* = 6); (6) cerulein + AN1284 0. 5 mpk group: mice were treated hourly (×4) with cerulein (50 µg/kg, in saline, IP, 0.2 ml)

and received AN1284 (0.5 mg/kg, SC, 0.2ml) 30 min post the first cerulein administration (*n* = 6); (7) cerulein + RvE-1 group: mice were treated hourly (×4) with cerulein (50 μg/kg, in saline, IP, 0.2 ml) and received AN1284 (1 mg/kg, SC, 0.2ml) 30 min post the first cerulein administration (*n* = 6);

Mice were killed by exsanguination at 4 hours after the first cerulein injection. Blood samples were obtained by direct intracardiac puncture. Pancreas were removed immediately and either flash frozen in liquid nitrogen, and stored at −80°C until assayed, or fixed in formaldehyde for histologic and immunohistochemical examination.

3.1.1 ***Test article Administration*** Test articles were dissolved in sterile saline (0.9% NaCl) to prepare a 10 mg/ml stock solutions. These stock solutions were further diluted in sterile saline to appropriate final concentrations before administration. In all groups, test articles were administered subcutaneously (SC) in a total volume of 0.2 ml, 30 min post the first cerulein IP injection.

3.1.2 ***End points*** Primary: serum amylase. Secondary: Pancreas histology; TNF- α, MPO, 3-NT and IκB-α.

4 METHODS

4.1 Serum Amylase Activity Measurements

Serum amylase activity was determined using a colorimetric assay kit (ab102523, Abcam). The values of serum amylase activity are expressed as units per liter (U/l).

4.2 Western Blot Analysis

Pancreas tissues from each mouse were suspended in extraction Buffer A containing 0.2 mM PMSF, 0.15 mM pepstatin A, 20 mM leupeptin, 1 mM sodium orthovanadate, homogenized at the highest setting for 2 min, and centrifuged at 12,000 rpm for 4 min at 4 °C. Supernatants represented the cytosolic fraction. The pellets, containing enriched nuclei, were resuspended in Buffer B containing 1%Triton X-100, 150mMNaCl, 10mM Tris–HCl pH 7.4, 1mMEGTA, 1mMEDTA, 0.2 mM PMSF, 20 mm leupeptin, and 0.2 mM sodium orthovanadate. After centrifugation 10 min at 12,000 rpm at 4 °C, the supernatants contained the nuclear protein content. Protein concentrations were estimated by the Bio-Rad protein assay using bovine serum albumin as standard. Briefly, samples were heated to 100 °C for 5 min, and equal amounts of protein were separated on 18 % SDS-PAGE gel and transferred to nitrocellulose membrane. Specific primary antibody, anti-IκB-α (1:1000; Santa Cruz Biotechnology, Inc.), was mixed in 1× PBS, 5 % w/v nonfat dried milk, 0.1 % Tween-20 (PMT), and incubated at 4 °C, overnight. Next, membranes were incubated with peroxidase-conjugated bovine anti-mouse IgG secondary antibody or peroxidase-conjugated goat anti-rabbit IgG (1:2000, Jackson ImmunoResearch) for 1 h at room temperature. To ascertain that blots were loaded with equal amounts of protein lysates, they were also incubated in the presence of the

antibody against β-actin (1:1000; Santa Cruz Biotechnology). Signals were detected with enhanced chemiluminescence detection system reagent according to manufacturer's instructions (SuperSignalWest Pico Chemiluminescent Substrate, Pierce). The relative expression of the protein bands was quantified by densitometry with Bio-Rad ChemiDoc™XRS+software and standardized to β-actin levels. Images of blot signals (8-bit/600-dpi resolution) were imported to analysis software (Image Quant TL, v2003). A preparation of commercially available molecular weight markers made of proteins of molecular weight 10 to 250 kDa was used to define molecular weight positions and as reference concentrations for each molecular weight.

4.3 Immunohistochemistry and Densitometric analysis

Immunohistochemistry (IHC) - Formalin fixed paraffin-embedded (FFPE) pancreas sections (7-µm) were used for immunohistochemical localization of MPO, Nitrotyrosine and TNF-a. Endogenous peroxidase in de-paraffinized sections was quenched with 0.3% (v/v) hydrogen peroxide in 60% (v/v) methanol for 30 min. The sections were made permeable by incubation with 0.1% (w/v) Triton X-100 in PBS for 20 min. Non-specific adsorption was minimized by incubating the sections in 2% (v/v) normal goat serum in PBS for 20 min. Endogenous biotin and avidin binding sites were blocked by sequential incubation for 15 min with biotin and avidin, respectively. Sections were then incubated overnight at 4°C with anti-mouse TNF- α rabbit polyclonal antibody (Santa Cruz Biotechnology, 1:200 in PBS, v/v) or with anti-mouse MPO rabbit polyclonal antibody (Santa Cruz Biotechnology, 1:200 in PBS, v/v), or with anti-mouse Nitrotyrosine rabbit polyclonal antibody (Santa Cruz Biotechnology, 1:500 in PBS, v/v). Then, the sections were washed with PBS, and incubated with a biotin-conjugated goat anti-rabbit IgG secondary antibody followed by an avidin-biotin peroxidase complex (Vector Laboratories, Milan, Italy). The immunoreactions were visualized by incubating the sections four minutes in a 0.1% 3, 3'-diaminobenzidine (DAB) and 0.02% hydrogen peroxide solution (DAB substrate kit; Vector Laboratories).

The immunohistochemical pictures were collected by Zeiss microscope using Axio Vision software. For graphic representation of densitometric analysis, we measured the intensity of positive staining (brown staining) by computer-assisted color image analysis (Leica QWin V3, UK). The percentage area of immunoreactivity (determined by the number of positive pixels) was expressed as percent of total tissue area (red staining).

4.4 Statistics

All values in the figures and text are expressed as mean ± standard error (S.E.M.) of the mean of n observations, where n represents the number of animals studied. The results were analyzed by one-way ANOVA followed by a Bonferroni post-hoc test for multiple comparisons. A p-value less than 0.05 is considered significant.

5 STUDY RESULTS

5.1 The effect of an AN1297 and AN1284 on serum amylase

Acute pancreatitis is a process of acute inflammation of the pancreas, with variable involvement of regional tissues or organ systems. The injection of cerulein, a cholecystokinin analog and pancreatic secretagogue, is a model with mild severity widely used. Hyperstimulation with cerulein leads to edematous pancreatitis characterized by a higher serum amylase level, edema, leukocyte infiltration, and vacuolation of acinar cells. In turn, there is an increased expression and release of pro- inflammatory cytokines and chemokines such as interleukin (IL)-1β, IL-6, monocyte chemotactic protein-1, tumor necrosis factor (TNF)-α, and IL-8 that are not normally detected in pancreas at high levels.

In this study, we used several of the above mentioned biomarkers to determine the extent of pancreatic injury following cerulein administration, and the therapeutic effect of AN1284 and AN1297. Serum amylase increased by an average of 7-fold, following cerulein injections. AN1284 had no significant effect on the levels of amylase measured in the serum (Figures 1,2). However, AN1297 significantly decreased serum amylase levels detected in a dose dependent manner, exhibiting a 73% and an 86% percent improvement over the vehicle control group, for 1 mg/kg and 2 mg/kg, respectively (Figures 1,2; Tables 1,2). The extent of improvement with AN1297 at both doses used, was greater than that of the positive control, resolvin E1 (RvE-1).

Fig. 1: AN1297 at 1 mg/kg significantly reduces serum amylase (Experiment I). Mice (*n*=10) were subjected to four hourly IP administrations of saline or 50 µg/kg cerulein. Thirty min post the first cerulein injection, the animals received a subcutaneous administration of either saline, 1 mg/kg AN1297, or 1 mg/kg AN1284. Four hours post the initiation of cerulein administration, animals were anesthetized, blood was collected and serum prepared. Serum amylase activities were determined for each mouse using a commercial kit, averaged and plotted. Error bars represent standard error.

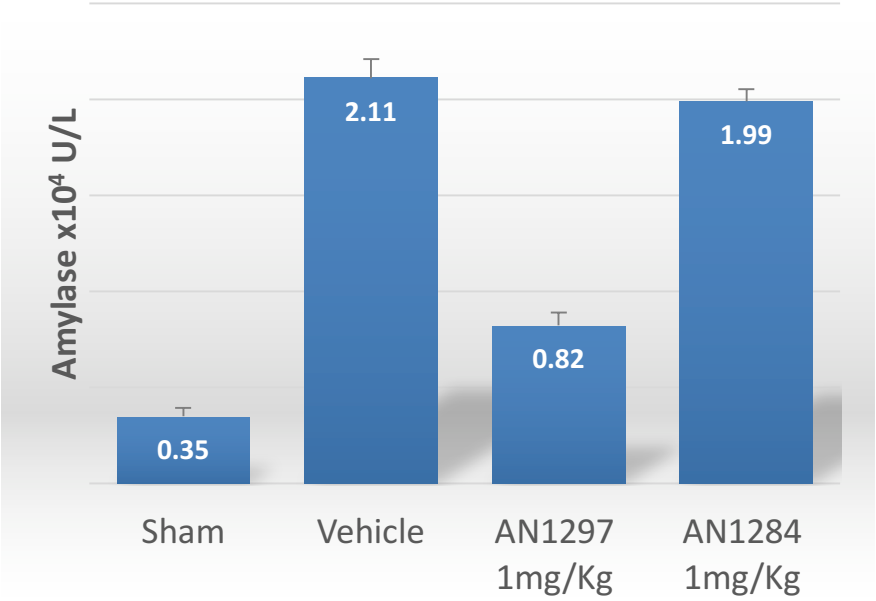


Table 1: Experiment I- Serum Amylase Activity Values [X10⁴U/L]

N°	Sham	Vehicle	AN1297 1mg/Kg	AN1284 1mg/Kg
1	0.2	2.5	1.2	2.3
2	0.5	1.5	0.9	1.9
3	0.3	2.3	0.7	2.0
4	0.5	2.2	0.5	1.8
5	0.2	1.9	0.7	1.9
6	0.3	2.1	0.8	2.1
7	0.5	2.3	1.0	2.0
8	0.5	1.8	0.9	1.7
9	0.3	2.0	0.7	1.9
10	0.2	2.5		2.3
N=	10	10	9	10
Mean	**0.35**	**2.11**	**0.82**	**1.99**
SD	0.135	0.318	0.205	0.197
SE	0.043	0.100	0.068	0.062
p value *vs* Sham		3.88E-12		
p value *vs* Vehicle			9.21E-09	0.324

Fig. 2: AN1297 at 2 mg/kg significantly reduces serum amylase (Experiment II). Mice (*n*=6) were subjected to four hourly IP administrations of saline or 50 µg/kg cerulein. Thirty min post the first cerulein injection, the animals received a subcutaneous administration of either saline, 0.5 or 2 mg/kg AN1297, or 0.25 or 0.5 mg/kg AN1284. Four hours post the initiation of cerulein administration, animals were anesthetized, blood was collected and serum prepared. Serum amylase activities were determined for each mouse using a commercial kit, averaged and plotted. Error bars represent standard error.

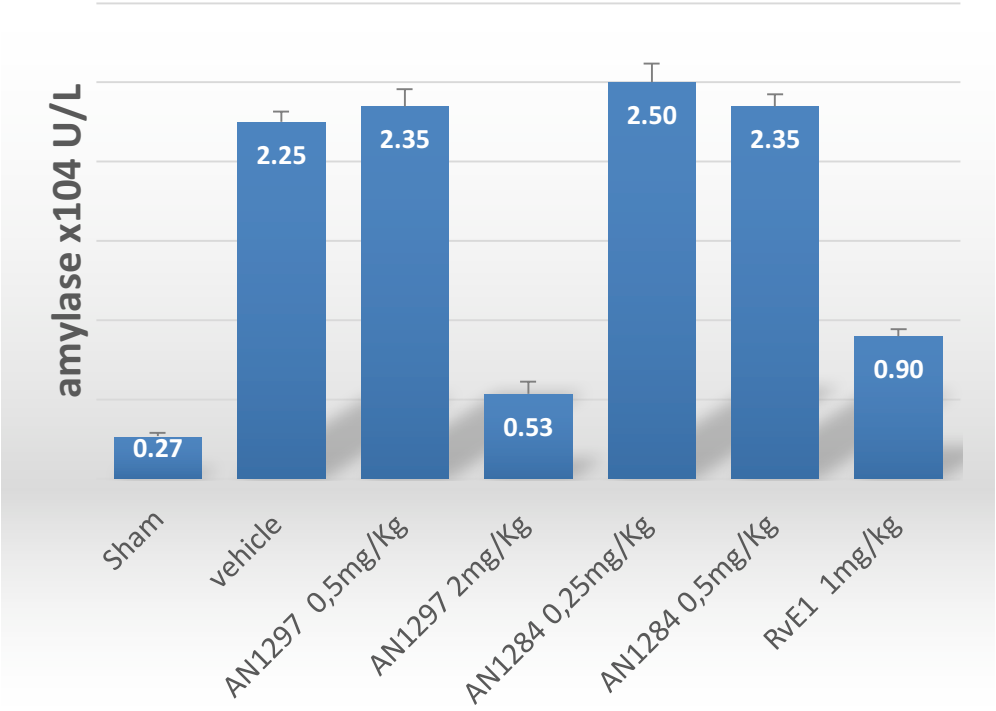


Table 2: Experiment II- Serum Amylase Activity Values [X10⁴U/L]

N°	Sham	vehicle	AN1297 0.5 mg/Kg	AN1297 2 mg/Kg	AN1284 0.25 mg/Kg	AN1284 0.5 mg/Kg	RvE1 1 mg/kg
1	0.2	2.2	2.2	0.5	2.5	2.3	0.9
2	0.3	2.0	2.2	0.9	2.9	2.6	0.9
3	0.3	2.3	2.5	0.3	2.2	2.4	0.7
4	0.3	2.2	2.5	0.5	2.8	2.5	0.8
5	0.2	2.5	2.0	0.5	2.3	2.2	1.0
6	0.3	2.3	2.7	0.5	2.3	2.1	1.1
N=	6	6	6	6	6	6	10
Mean	**0.27**	**2.25**	**2.35**	**0.53**	**2.50**	**2.35**	**0.90**
SD	0.052	0.164	0.259	0.197	0.290	0.187	0.141
SE	0.021	0.067	0.106	0.080	0.118	0.076	0.045
p value vs Sham		7.29E-11					
p value vs Vehicle			0.443	1.47E-08	0.096	0.348	2.98E-08

5.2 *The effect of an AN1297 on markers of inflammation*

The extent of inflammatory response and pancreatic injury associated with cerulein administration was evaluated in both experiments by measuring the levels of the pro-inflammatory cytokine TNF-α and the downstream inhibitor IκB-α. In Addition, neutrophil infiltration was evaluated by measuring the levels of myeloperoxidase (MPO) and

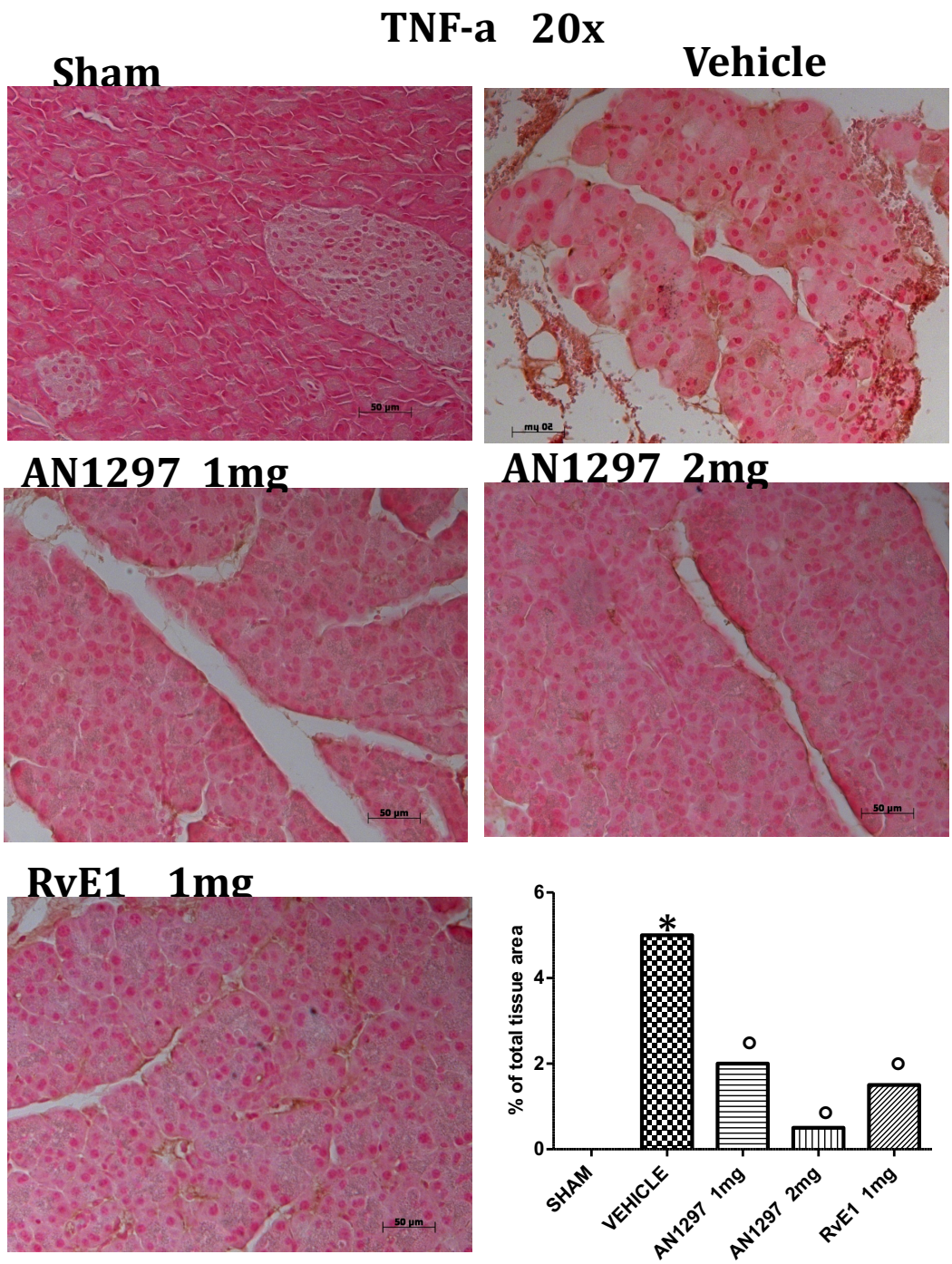
nitrotyrosine (3-NT) staining was used to assess peroxinitrite formation. H&E staining was used to provide a histological evaluation of the extent of pancreatic edema, necrosis, and neutrophil infiltration. For these analyses, pancreatic tissues (collected in both experiments, I+II) embedded in formalin, were used for histologic analysis and immunohistochemistry stainings, whereas snap frozen tissues kept at -80^{0}C were used for western blot analysis.

Cerulein administration induced a dramatic increase in the levels of TNF-α detected in pancreatic tissue of mice from the vehicle control group (Table 3, Figure 3). AN1297 induced a dose dependent, significant decrease in TNF-α levels, resulting in levels close to those scored in the sham group (Table 3, Figure 3).

Table 3: TNF-α densitometry, expressed as % of total tissue area

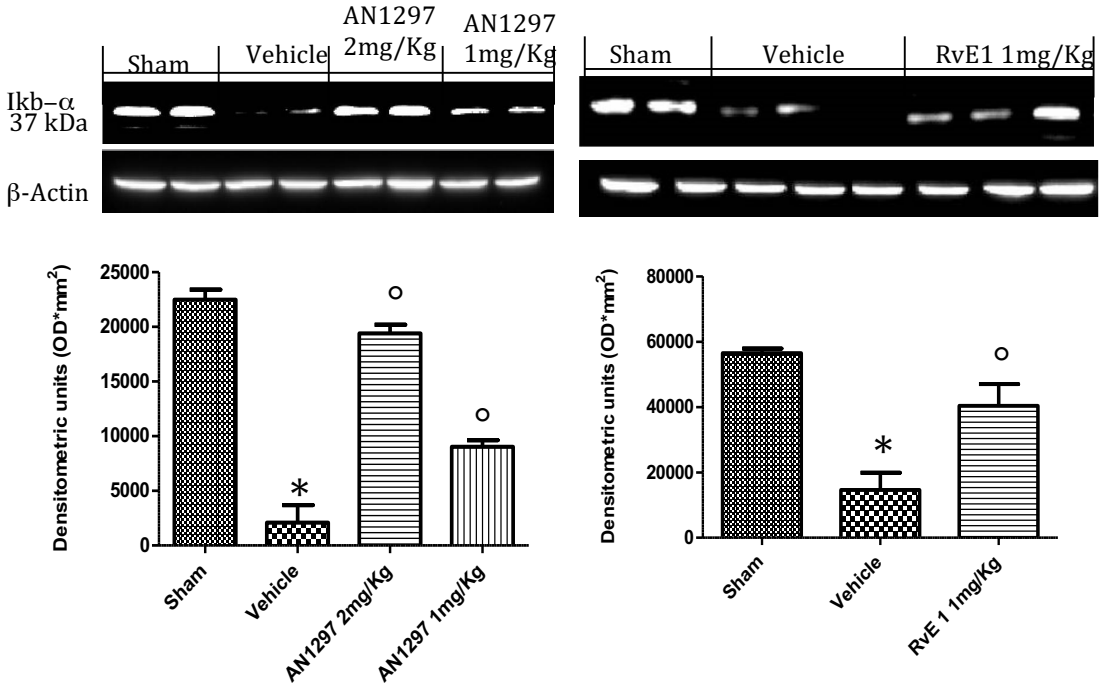
	N°	Sham	Vehicle	AN1297 1 mg/Kg	AN1297 2 mg/Kg	RvE-1 1 mg/kg
Exp. I	1	0	3	2.5		1.5
	2	0	6	3.0		1.6
	3	0	6	0.5		1.4
Exp. II	4	0	4		0.4	
	5	0	6		0.5	
	6	0	5		0.6	
	N=	6	6	3	3	3
	Mean	**0.00**	**5.00**	**2.00**	**0.50**	**1.50**
	SD	0	1.265	1.323	0.1	0.1
	SE	0	0.516	0.764	0.0577	0.0577
p value vs Sham			2.13E-06			
p value vs Vehicle				0.013	5.73E-04	0.002

Fig. 3: AN1297, at 1 and 2 mg/kg, significantly reduces TNF-α levels (Experiment I+II). Slices of formalin embedded pancreases, obtained in the two experiments, were stained for TNF-α and quantified, as described in the methods. Representative slides are shown.



On the other hand, the detected levels of the downstream cellular inhibitor, IκB-α, was significantly decreased following pancreatitis induction with cerulein (Fig. 4). AN1297 dose-dependently increased the levels of IκB-α, almost restoring sham levels (Fig. 4).

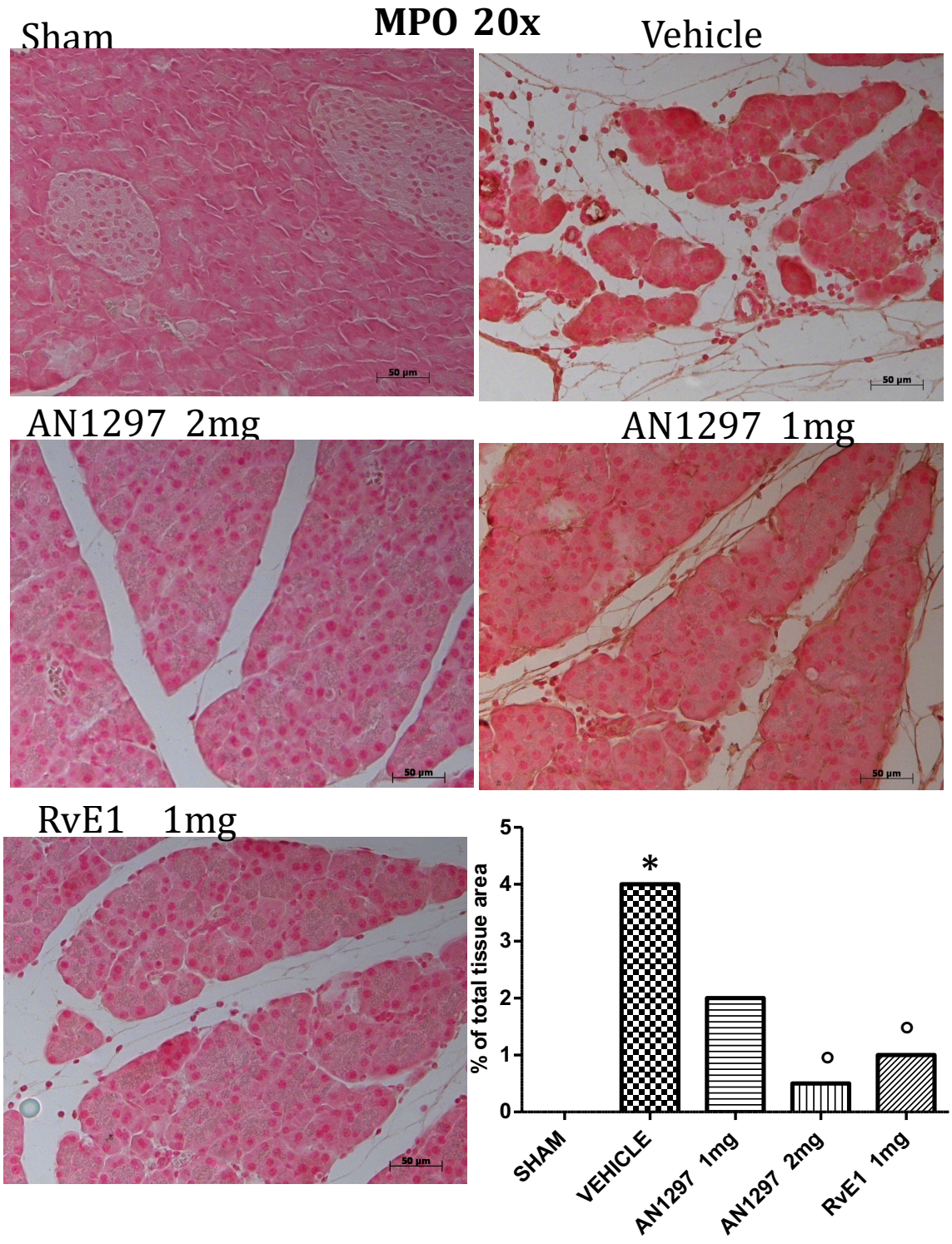
Fig. 4: AN1297, at 1 and 2 mg/kg, significantly increases IκB-α levels (Experiment I+II). Snap frozen pancreatic tissues, obtained in the two experiments, were homogenized and analyzed by a western blot for IκB-α and quantified, as described in the methods.



Neutrophil infiltration and peroxinitrite formation, two pronounce markers of inflammation, were evaluated by measuring the levels of myeloperoxidase (MPO) and nitrotyrosine (3-NT), respectively. The levels of both markers were dramatically increased upon cerulein injections, but were significantly reduced by treatment with AN1297, in a dose-dependent manner (Fig. 5, Table 4).

Fig. 5: AN1297, at 1 and 2 mg/kg, significantly reduces MPO and 3-NT levels (Experiment I+II). Slices of formalin embedded pancreases, obtained in the two experiments, were stained for myeloperoxidase (MPO, a) and nitrotyrosine (3-NT, b) and quantified, as described in the methods. Representative slides are shown.

a


MPO 20x
Sham
Vehicle
AN1297 2mg
AN1297 1mg
RvE1 1mg
50 µm
% of total tissue area
0
1
2
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4
5
*
°
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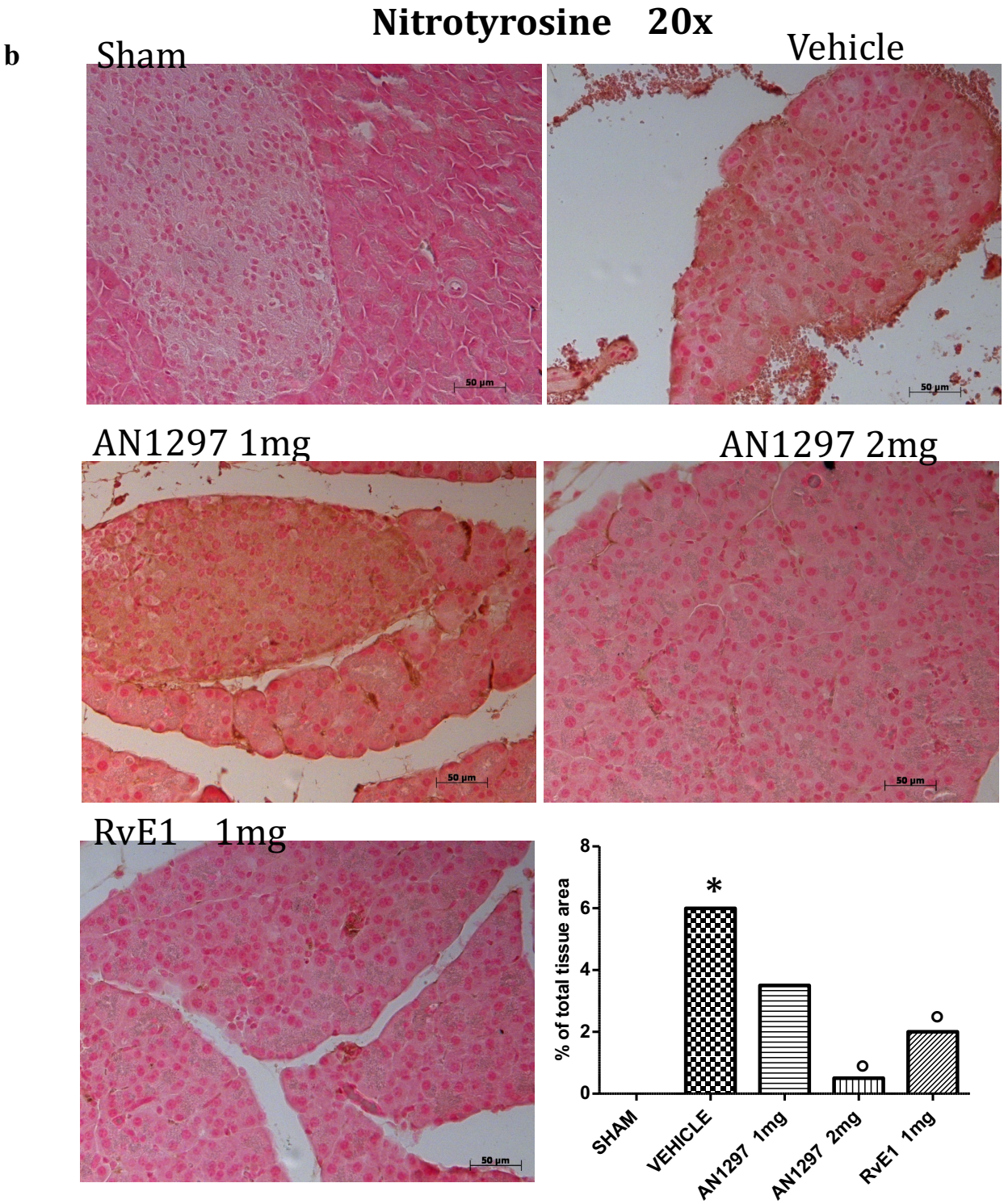
SHAM
VEHICLE
AN1297 1mg
AN1297 2mg
RvE1 1mg

b


Nitrotyrosine 20x
Sham
Vehicle
AN1297 1mg
AN1297 2mg
RvE1 1mg
50 µm
% of total tissue area
8
6
4
2
0
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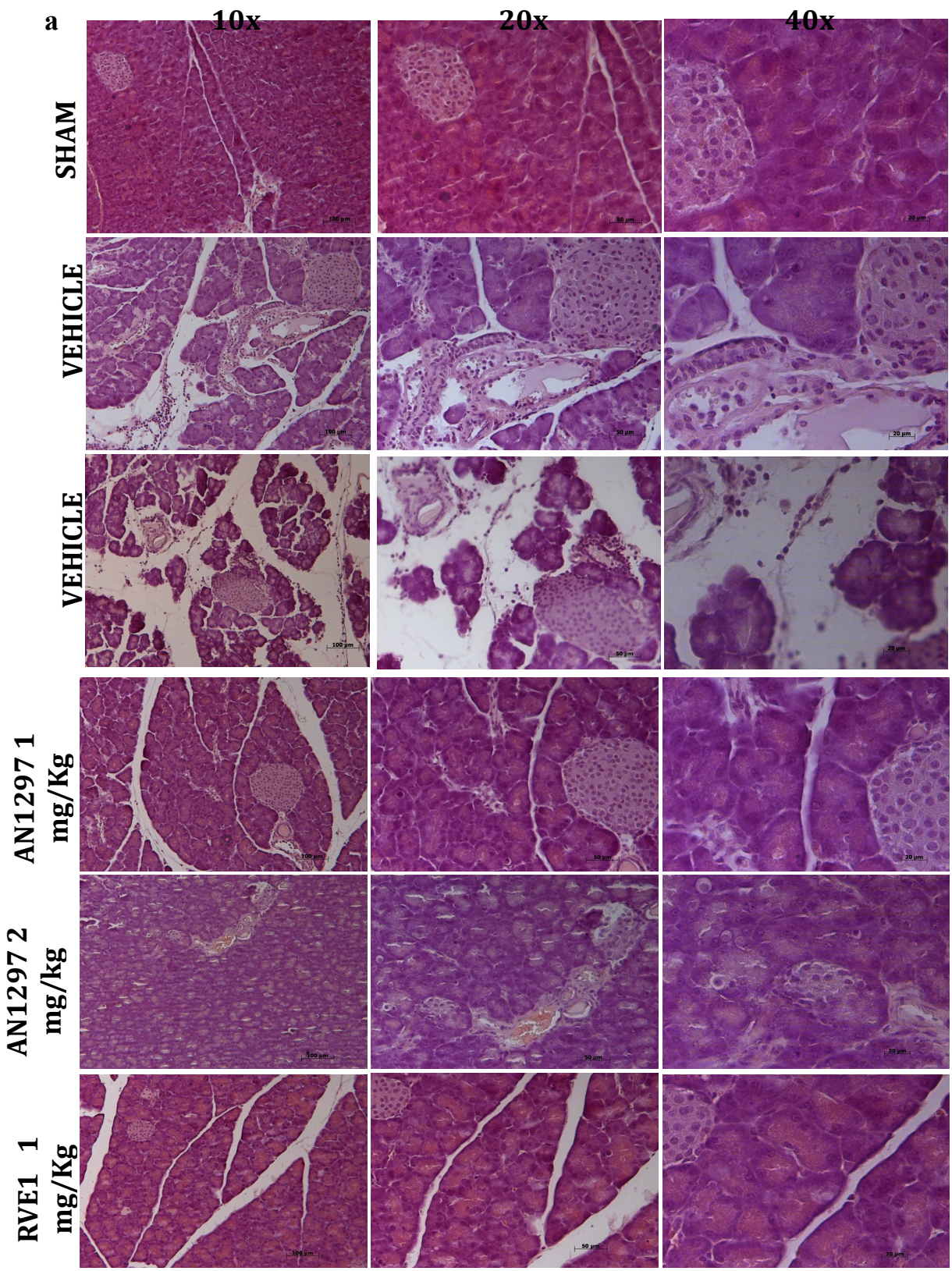
o
o
SHAM
VEHICLE
AN1297 1mg
AN1297 2mg
RvE1 1mg

Table 4: Densitometry values of myeloperoxidase (MPO) and nitrotyrosine (3-NT), expressed as % of total tissue area.

	N°	Sham	vehicle	AN1297 1mg/Kg	AN1297 2mg/Kg	RvE1 1mg/kg
MPO	1	0	5	1.5		1.0
	2	0	4	2.0		1.1
	3	0	5	2.5		0.9
	4	0	3		0.4	
	5	0	5		0.7	
	6	0	2		0.5	
	N=	6	6	3	3	3
	Mean	**0.00**	**4.00**	**2.00**	**0.50**	**1.00**
	SD	0	1.265	0.5	0.15	0.1
	SE	0	0.516	0.289	0.087	0.058
p value vs Sham			1.56E-05			
p value vs Vehicle				0.037	2.43E-03	0.005
3-NT	1	0	6	4.0		2.1
	2	0	7	5.0		2.0
	3	0	4	1.5		1.9
	4	0	5		0.4	
	5	0	8		0.6	
	6	0	6		0.5	
	N=	6	6	3	3	3
	Mean	**0.00**	**6.00**	**3.50**	**0.50**	**2.00**
	SD	0	1.414	1.803	0.096	0.1
	SE	0	0.577	1.041	0.055	0.058
p value vs Sham			1.12E-06			
p value vs Vehicle				0.055	3.32E-04	0.002

Neutrophil infiltration was also assessed by histological evaluation, using H&E staining, together with scoring of pancreatic edema and necrosis. The tissues retrieved from the vehicle-control treated groups, exhibited histological scores that indicate mild injuries (Fig. 6, Table 5). AN1297 caused a dose-dependent decrease in all scores (Fig. 6, Table 5).

Fig. 6: AN1297, at 1 and 2 mg/kg, significantly reduces histological injury score (Experiment I+II). Slices of formalin embedded pancreases, obtained in the two experiments, were stained with H&E and quantified for edema, necrosis and neutrophil infiltration, as described in the methods. Representative slides are shown (a), error bars represent standard error (b).


a
10x
20x
40x
SHAM
VEHICLE
VEHICLE
AN1297 1 mg/Kg
AN1297 2 mg/kg
RVE1 1 mg/Kg

b

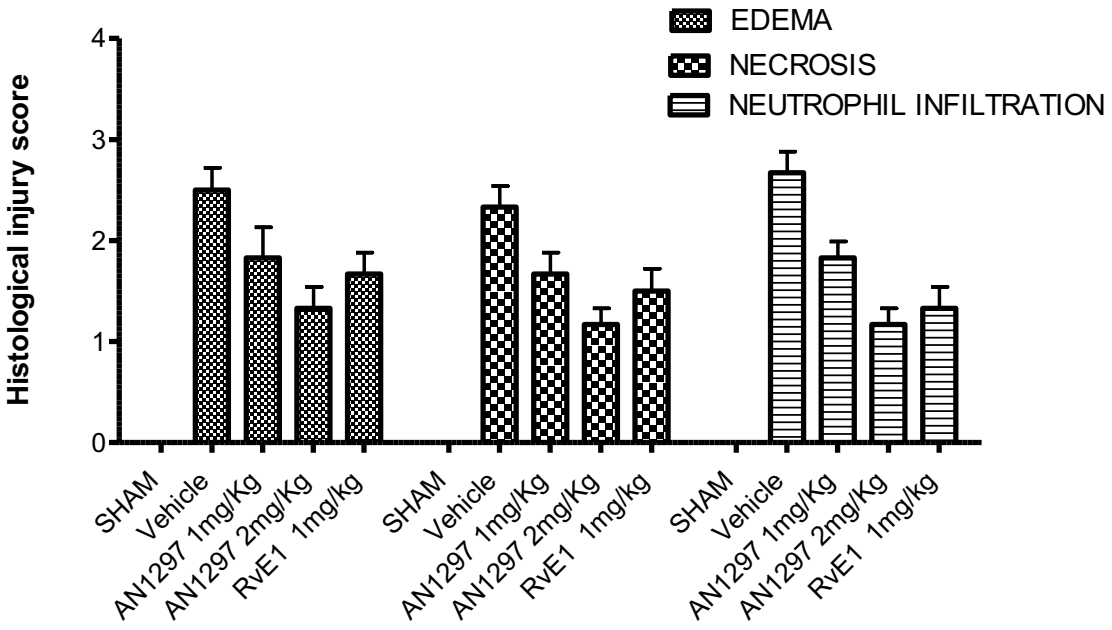

EDEMA
NECROSIS
NEUTROPHIL INFILTRATION
Histological injury score
0
1
2
3
4
SHAM
Vehicle
AN1297 1mg/Kg
AN1297 2mg/Kg
RvE1 1mg/kg
SHAM
Vehicle
AN1297 1mg/Kg
AN1297 2mg/Kg
RvE1 1mg/kg
SHAM
Vehicle
AN1297 1mg/Kg
AN1297 2mg/Kg
RvE1 1mg/kg

Table 5: Histological scores.

	N°	Sham	vehicle	AN1297 1 mg/Kg	AN1297 2 mg/Kg	RvE1 1 mg/kg
EDEMA	1	0	2	3	1	2
	2	0	3	2	1	2
	3	0	2	2	1	2
	4	0	3	1	2	1
	5	0	3	2	1	2
	6	0	2	1	2	1
	N=	6	6	6	6	6
	Mean	**0.00**	**2.50**	**1.83**	**1.33**	**1.67**
	SD	0	0.5477	0.7528	0.5164	0.5164
	SE	0	0.2236	0.3073	0.2108	0.2108
p value vs Sham			5.67E-07			
p value vs Vehicle				0.110	3.51E-03	0.022
NECROSIS	1	0	2	2	1	1
	2	0	3	2	1	2
	3	0	2	2	1	2
	4	0	2	1	1	1
	5	0	3	2	1	2
	6	0	2	1	2	1
	N=	6	6	6	6	6
	Mean	**0.00**	**2.33**	**1.67**	**1.17**	**1.50**
	SD	0	0.516	0.516	0.408	0.548
	SE	0	0.211	0.211	0.167	0.224
p value vs Sham			6.23E-07			
p value vs Vehicle				0.049	1.46E-03	0.022
NEUTROPHIL INFILTRATION	1	0	3	2	1	1
	2	0	3	2	1	2
	3	0	2	2	1	1
	4	0	3	2	1	1
	5	0	3	2	1	2
	6	0	2	1	2	1
	N=	6	6	6	6	6
	Mean	**0.00**	**2.67**	**1.83**	**1.17**	**1.33**
	SD	0	0.516	0.408	0.408	0.516
	SE	0	0.211	0.167	0.167	0.211
p value vs Sham			1.78E-07			
p value vs Vehicle				0.011	2.34E-04	0.001

6 STUDY CONCLUSIONS

This study was designed to provide an initial assessment of a possible therapeutic role of AN1297 and AN1284 under acute pancreatitis conditions, caused by injection of cerulein. All examined parameters showed a marked difference upon cerulein administration, in comparison to sham control animals. The primary indication of efficacy was measuring the levels of serum amylase, which is released into the blood when the pancreas is

inflamed or diseased. AN1284 had no significant effect on the highly increased amylase levels detected following pancreatitis induction. AN1297, on the other hand, had a significant and dose-dependent effect on serum amylase, as well as on all other tested parameters indicative of inflammation: TNF-α, IκB-α, neutrophil infiltration (MPO levels and histology), peroxinitrite formation (3-NT), and histological scores of pancreatic edema and necrosis. AN1297 at doses of 1 and 2 mg/kg showed improvements of 73% and 86%, respectively, in serum amylase levels; 60% and 90%, respectively in TNF-α levels; 50% and 87.5%, respectively, in MPO levels and 42% and 92%, respectively, in 3-NT levels. In addition, histological scores were improved by an average of 29% with 1 mg/kg AN1297 and by an average of 51% with a treatment of 2 mg/kg AN1297. In all cases, the higher dose of AN1297 (2 mg/kg) demonstrated a better efficacy than the positive control used in these experiments: 1 mg/kg RvE-1.

Whereas AN1297 demonstrated a significant effect on short-term inflammatory response biomarkers, it is intriguing to assess its long term effect on survival in this model of acute pancreatitis.