

Mechanistic Insights into Ferroptotic Cell Death in Pancreatic Islets

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ABSTRACT

Ferroptosis was recently identified as a non-apoptotic, iron-dependent cell death mechanism that is involved in various pathologic conditions. There is first evidence for its significance also in the context of islet isolation and transplantation. Transplantation of pancreatic human islets is a viable treatment strategy for patients with complicated diabetes mellitus type 1 (T1D) that suffer from severe hypoglycemia. A major determinant for functional outcome is the initial islet mass transplanted. Efficient islet isolation procedures and measures to minimize islet loss are therefore of high relevance. To this end, better understanding and subsequent targeted inhibition of cell death during islet isolation and transplantation is an effective approach. In this study, we aimed to elucidate the mechanism of ferroptosis in pancreatic islets. Using a rodent model, isolated islets were characterized relating to the effects of experimental induction (RSL3) and inhibition (Fer1) of ferroptotic pathways. Besides viability, survival, and function, the study focused on characteristic ferroptosis-associated intracellular changes such as MDA level, iron concentration and the expression of ACSL4. The study demonstrates that pharmaceutical induction of ferroptosis by RSL3 causes enhancement of oxidative stress and leads to an increase of intracellular iron, zinc and MDA concentration, as well as the expression of ACSL4 protein. Consequently, a massive reduction of islet function, viability, and survival was found. Fer1 has the potential to inhibit and attenuate these cellular changes and thereby protect the islets from cell death.

ABBREVIATIONS

ACSL 4	Long-chain-fatty-acid CoA ligase 4
DAPI	4',6-Diamidino-2-phenylindole
DMSO	Dimethyl sulfoxide
DTZ	Dithizone
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmatic reticulum
FDA	Fluorescein diacetate
Fer-1	Ferostatin 1
GSIS	Glucose-stimulated insulin secretion
HPLC	High-performance liquid chromatography
ICP-MS	Inductively-coupled plasma mass spectrometry
IEQ	Islet equivalent
IP	Islet particals
MDA	Malondialdehyde
MKRB	Modified Krebs–Ringer buffer
PBS	Phosphate buffered saline
PFA	Paraformaldehyde
PI	Propidium iodide
RSL 3	Ras-selective lethal 3
SI	Stimulation index
T1D	Type 1 diabetes
TBA	Thiobarbituric acid
TUNEL	TdT-mediated-dUTP-biotin nick end labeling

Introduction

Ferroptosis was identified as a non-apoptotic cell death mechanism, which causes iron-dependent peroxidation of membrane lipids and subsequent membrane rupture [1, 2]. As a form of necroptotic cell death, ferroptosis has already been shown to play an important role in the pathogenesis of various diseases [3]. Ferroptosis can be experimentally induced by the glutathione peroxidase-4 (GPX4) inhibitor RSL3. The inhibition of GPX4 causes oxidative stress through the release of free iron molecules and induction of lipid peroxidation, followed by a membrane burst with massive necrotic inflammation [1, 4]. The effects of ferroptotic cell death can be attenuated by treatment with ferostatin-1 (Fer-1) [5, 6], a small lipophilic molecule with various functions. Fer-1 mainly acts as an iron scavenger and prevents lipid peroxidation and subsequent membrane rupture [5].

The transplantation of pancreatic islets into the liver represents a therapeutic strategy mainly for patients with type 1 diabetes (T1D) experiencing life-threatening hypoglycemic situations despite optimal diabetes therapy or, in the case of an autologous transplantation setting, to prevent patients undergoing (sub-)total pancreatectomy from iatrogenic diabetes [5, 7, 8]. The intraportal transplantation of pancreatic islets is less invasive than a complete pancreas transplantation but has comparable effects on the stabilization of glycemic control and diabetes-associated complications [8, 9]. However, it is estimated that nearly 70% of the islet graft is lost during the peri- and post-transplant period [10].

To further improve the outcome of this therapy, it is of key importance to minimize islet loss and consequently improve islet functional potency and survival. A better understanding of mech-

anisms mediating islet death during and after the isolation process and targeted prevention are therefore key issues. Bruni et al. first described in 2018 that human pancreatic islets are susceptible to pharmaceutically induced ferroptosis. The aim of our study was to gain closer insight into the mechanism of ferroptosis in pancreatic islets on a cellular and functional level and thereby identify potential interventional strategies.

Materials and Methods

Islet isolation

Pancreatic islets were isolated from female Wistar rats according to guidelines established by the University of Dresden Institutional Animal Care and Use Committee. Animals were euthanized with CO₂. Afterwards, the abdomen was opened, and the pancreatic duct was clamped at the papilla. The digestion solution [1 mg/ml collagenase V (Sigma-Aldrich) and 100 µg/ml DNase (Roche)] was injected in situ into the pancreas via the bile duct. The pancreas was carefully dissected and transferred to digestion solution. The digestion was supported by gentle shaking at 37 °C for approximately 12 minutes. After adding cold washing solution (RPMI 5.5 mM glucose, 10 % FBS), the digest was filtered through a cell strainer with a pore size of 600 µm (Sigma-Aldrich) and centrifuged for 1 min (277 g, Acc6, Dec6, 4 °C). The washing procedure was repeated three times. Pancreatic islets were then separated from exocrine tissue by discontinuous density gradient centrifugation (15 min, 1590 g, Acc2, Dec2, 4 °C) using Ficoll (Sigma-Aldrich) density layers of 1.125 g/cm³, 1.096 g/cm³, 1.08 g/cm³, and 1.06 g/cm³. The interfaces containing the purified islets were collected, washed and cultured in 5.5 mM glucose RPMI 1640 (PAA) supplemented with 10 % FBS, 20 mM HEPES, 1 × penicillin-streptomycin at 37 °C in a 5 % CO₂ atmosphere prior to further experimentation.

Islet yield and purity

Dithizone (DTZ), a zinc chelating agent, is known to selectively stain the islets of Langerhans in the pancreas. While exocrine tissue is not stained, the endocrine part appears red. For determination of the islet yield, islet particle number (IP) and islet equivalents (IEQ, islet volume normalized to 150 µm diameter) were determined. To this end, representative triplicate samples were stained with dithizone [2 % w/v DTZ in 0.25 % v/v DMSO in phosphate-buffered saline (PBS)] and examined using an inverted microscope equipped with a 10 × objective and eyepiece micrometer. All islets with a diameter > 50 µm were grouped into diameter classes of 50 µm segments (i. e., 50–100, 100–150, 150–200, etc.). Each diameter class was converted into the mean volume of 150 µm diameter islets by a relative conversion factor. This method enables the evaluation of the total IEQ number and total IP number of each preparation. The purity and morphology were described descriptively using light microscopy observation at 100 × magnification [11].

Islet viability

Islet viability was assessed by double staining with fluorescein diacetate (FDA) and propidium iodide (PI). FDA is a non-fluorescent molecule, which is hydrolyzed to green-fluorescent fluorescein in live cells. Dead cells cannot accumulate or hydrolyze FDA. PI only

permeates through the membrane of dead cells resulting in red fluorescence. In detail, islets were washed with PBS and both agents, FDA and PI, were added at a final concentration of 0.5 and 75 μM , respectively. Samples were incubated in the dark for 5 minutes and evaluated using an inverted fluorescence microscope (100 \times magnification). For each sample, 100 islets were individually analyzed by calculating the percentage of non-viable cells (red) and viable cells (green). Islets were grouped into 5 categories (0%, 25%, 50%, 75%, and 100% viability) and islet viability was calculated.

Glucose-stimulated insulin secretion

The functional secretory capacity of pancreatic islets was analyzed by glucose-stimulated insulin secretion. Therefore, islets were transferred to Modified Krebs Ringer Buffer (MKRB) and equilibrated at 3.3 mM glucose at 37 °C for 30 minutes. Afterwards, islets were divided into two groups containing fresh MKRB with either 3.3 mM or 16.7 mM glucose and incubated for 1 hour in a gently shaking water bath at 37 °C. For both conditions, five samples containing ten islets each were used. After incubation, islets were collected by gentle centrifugation at 200 g for 1 minute. Secreted insulin in the supernatants was measured by ELISA (Mercodia Insulin Elisa Kit). Pellets were resuspended in 200 μl PBS and analyzed for DNA content determined by DNeasy Kit (Qiagen). Stimulation Index (SI) is calculated as the ratio of secreted insulin in high glucose in comparison to low glucose, both normalized to DNA content [$\text{SI} = (\text{Insulin}_{\text{stim}}/\text{total DNA}_{\text{stim}})/(\text{Insulin}_{\text{res}}/\text{total DNA}_{\text{res}})$].

Lipid peroxidation

Lipid peroxidation is the degradation of lipids that occurs as a result of oxidative damage, typically by reactive oxygen species, and is a useful marker for oxidative stress. The peroxidation process leads to the production of malondialdehyde (MDA), which can be measured using the Malondialdehyde Microplate Assay Kit (Cohesion Biosciences). The lipid peroxidation was determined by the reaction of MDA with thiobarbituric acid (TBA) to generate the MDA-TBA adduct. The MDA-TBA adduct was quantified colorimetrically ($\lambda = 532 \text{ nm}$ and 600 nm). MDA levels were calculated according to the manufacturer's instruction.

Zinc and iron measurement

For the Inductively coupled plasma mass spectrometry analysis (ICP-MS), the islets were thawed and centrifuged. The supernatant was discarded, and the pellet resuspended in 200 μl 50 μM NaOH. For the physical cell lysis, the islets were drawn up six times with a syringe and spread out again. Then 50 μl of each sample was used for protein quantification. The remaining 150 μl was placed in a concentrator at 60 °C for 4 hours. Afterwards, 200 μl HNO_3 was added to resuspend the cell extract by thorough vortexing. To complete the digestion, the samples were heated for further 4 hours at 80 °C. After digestion, the samples were cooled to room temperature overnight. An amount of 2.6 ml of HPLC water was added to the samples and the iron concentration was analyzed by ICP-MS.

Islet treatment with Fer-1 over a period of 7 days

After isolation, all islets were aliquoted equally into two treatment groups: (i) culture media with vehicle (DMSO) as solvent control and

(ii) culture media with 10 μM Fer-1 (Ferrostatin-1, Merck Millipore, 341494). Readout assays were performed on days 3, 5, and 7.

RSL3 dilution series

After isolation, all islets were aliquoted equally into five groups with increasing concentrations of RSL3 (5 μM , 10 μM , 15 μM , 20 μM , 40 μM ; RSL3, type 2 FIN, Selleck Chemicals, S8155) and a solvent control with DMSO. After 24 hours, FDA/PI assay was performed, and viability was determined.

Ferroptosis inhibition

Islets were divided equally into four treatment groups: (i) culture media with vehicle (DMSO) as solvent control, (ii) culture media with a single treatment of 10 μM Fer-1 and day 0, (iii) culture media with a single treatment of 20 μM RSL3 on day 1, (iiii) culture media with a combination of 10 μM Fer-1 on day 0 and 20 μM RSL3 on day 1. The day of isolation was defined as day 0. All readouts were performed on day 2 after isolation.

Immunohistochemical analysis

For immunohistochemical analysis, 80–100 islets were fixed in 4% paraformaldehyde (PFA) for at least 1 hour, washed with PBS and embedded in tissue-Tek O.C.T. (Sakura Finetek). Embedded islets were sliced by cryosectioning in 6 μM thin sections onto microscope slides. Sections were rehydrated and washed with PBS. Antigen retrieval was performed with PBS with 3% Triton for 15 minutes. After blocking non-specific antibody binding sites with background sniper (Biocare Medical) for 11 minutes at room temperature, the sections were incubated at 4 °C with primary antibodies (Insulin/Glucagon/Somatostatin/ACSL4/TUNEL) diluted in PBS with 0.2% Triton, 2% BSA, and 2% goat serum overnight. Sections were washed with PBS in 0.5% Tween. Secondary antibodies together with 4',6-diamidino-2-phenylindole (DAPI) were diluted in PBS with 0.2% Triton, 2% BSA, and 2% goat serum. After incubation for 1 hour, the sections were finally washed with PBS and 0.5% Tween. Immunofluorescence microscopy was performed using Zeiss Axiovert200M with AxioCamMRC5.

Statistical Analysis

Statistical analysis was performed with GraphPad Prism 8. For comparison between groups, ordinary one-way-ANOVA ($p < 0.05$) test with Tukey's multiple comparison was used. Results are shown as mean \pm SEM from $n = X$ independent experiments.

Results

RSL3 causes massive islet death in a dose-dependent manner and pretreatment with Fer-1 can rescue islet viability and function

We observed that ferroptosis induction with increasing concentrations of RSL3 resulted in a significant reduction in islet viability (**► Fig. 1a**). At concentrations of 10 μM RSL3, islet viability was reduced to 40.3% compared to 77.3% in the control group. Induction of ferroptosis with 40 μM RSL3 had a fatal effect on islet viability. In addition, TUNEL-staining was performed to detect apoptotic cell death by labeling fragmented DNA and differentiate between

apoptotic and ferroptotic cell death (► Fig. 5a, vide infra). Treatment with 10 μM RSL3 and Fer-1 alone and in combination did not cause an increase in TUNEL positive cells compared to the control group.

The viability of islets challenged with 20 μM RSL3 could effectively be preserved by pretreatment with 10 μM Fer-1 for 24 hours (► Fig. 1b). Similar effects were observed regarding islet function (► Fig. 2). Pretreatment with Fer-1 increased functional potency compared to RSL3 induction alone. Interestingly, single treatment

with Fer-1 led to a reduction of islet stimulation Index (7.25 compared to 20.04 in the control group).

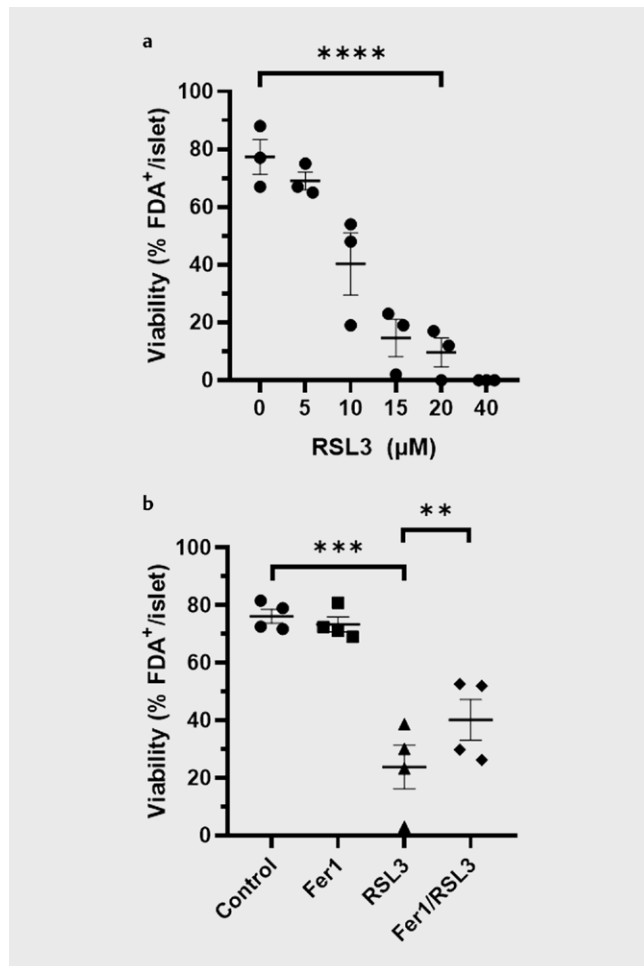
Treatment with Fer-1 alone did not impact on islet survival, viability or islet architecture

To determine whether ferroptotic cell death occurs as a common cell death mechanism in cultured rodent islets, the culture time was prolonged to seven days. We observed that treatment with Fer-1 alone over 7 days had no impact on islet viability (► Fig. 3). In order to prove whether the sustained islet viability is the result of a positive selection process during islet culture, or whether Fer-1 has a direct effect on the survival of the islets in culture, we examined cell survival over the period of seven days (quantification of IP; ► Fig. 3). Both groups showed a steady decrease in IP/ml without significant difference.

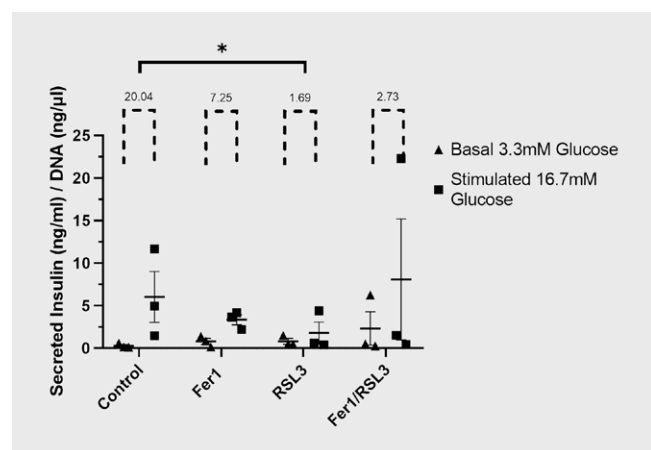
With immunohistochemical analysis we could demonstrate that the prolonged treatment with Fer-1 did not influence islet architecture (► Fig. 5b, vide infra).

RSL3 and Fer-1 influence the intracellular iron, and zinc concentration of cultured rodent islets

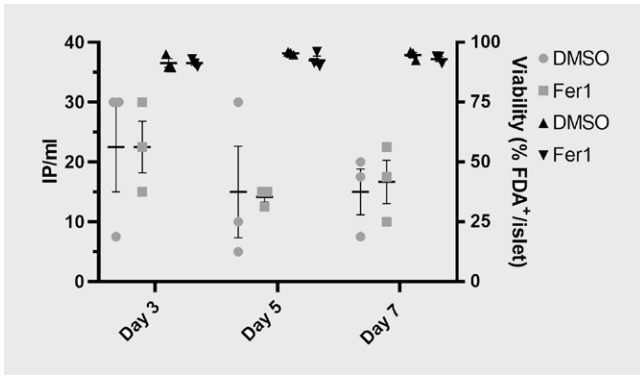
In order to elucidate intracellular changes during ferroptosis, the intracellular iron and zinc concentrations were measured. Culturing of the islets with the ferroptosis activator RSL3 increased the iron concentration to 0.88 ± 0.17 nmol/mg protein compared to 0.59 ± 0.05 nmol/mg protein (► Fig. 4a). Interestingly, pretreatment with the ferroptosis inhibitor Fer-1 could significantly reverse this effect by almost 50% and resulted in an iron concentration of 0.42 ± 0.03 nmol/mg protein. Furthermore, Fer1 treatment showed a slight reduction of the iron concentration in contrast to control. Several studies also describe ferroptosis induction by zinc, so we



► **Fig. 1** Islet viability expressed as FDA positive cells per islet after exposure to increasing concentrations of RSL3 (a) and treatment with RSL3 and Fer1 alone and in combination (b): a: Increasing RSL3 concentrations led to a dose-dependent reduction in viability. Islets were treated with increasing concentrations of RSL3 and viability was calculated as FDA+ cells/islet in percentage. Compared to the control group (77.30%), 10 μM RSL3 reduced the viability to 40.33% and 20 μM led to a viability of only 9.66%. Treatment with 40 μM RSL3 resulted in complete loss of viable cells. Data are presented as mean with single values from $n = 3$ independent experiments; $p < 0.005$. b: Ferrostatin has the potential to protect islets from the pathogenic metabolites produced by RSL3. Islets were treated with Fer1 (10 μM) and RSL3 (20 μM) alone and in combination and viability was calculated as FDA+ cells/islet in percent. Islet viability was significantly improved by pre-treatment with the ferroptosis inhibitor Fer1 (23.78% (RSL3) vs. 40.21% (Fer1/RSL3)). Data are presented as mean with single values from $n = 4$ independent experiments; $p < 0.05$.



► **Fig. 2** Islet functional capacity as measured by glucose stimulated insulin secretion (GSIS): Islets were treated with 10 μM Fer1 and 20 μM RSL3 alone and in combination. Insulin secretion was determined by GSIS assay, and the stimulation index (SI) was calculated. Islet function was significantly impaired by pharmaceutical induction of ferroptosis (SI of 1.69 in the RSL3 group compared to 20.04 in the control group). Simultaneous ferroptosis inhibition by Fer1 could attenuate this detrimental effect (SI of 2.73). Data are presented as mean with single values from $n = 3$ independent experiments; $p < 0.05$.



► **Fig. 3** Effect of the ferroptosis inhibitor Fer1 alone on islet viability without pharmaceutical ferroptosis induction: Islet viability (black) was not improved due to the treatment with Fer1 alone as DMSO control islets and treated islets maintained 90% viability over a period of seven days. Islet particles (grey) were determined to prove whether the stable viability was due to a positive selection process during culture or the result of Fer1 treatment. IP/ml showed a continuous decrease during the culture period. Data are presented as mean with single values from $n = 3$ independent experiments.

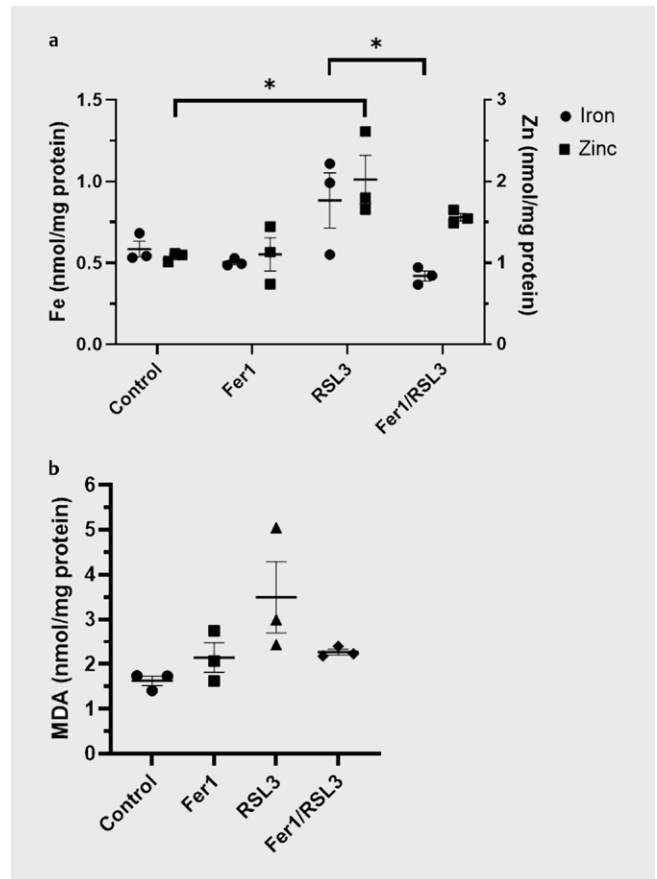
decided to measure intracellular zinc concentration in addition to intracellular iron concentration [12, 13].

Similar to the effect on iron concentrations, a treatment of the islets with RSL3 alone significantly increased the zinc concentration to 2.00 ± 0.30 nmol/mg protein in contrast to control islets with 1.10 ± 0.03 nmol/mg protein. A pretreatment of RSL3 challenged islets with Fer-1 could reduce the zinc concentration of the islets to 1.56 ± 0.05 nmol/mg protein (► **Fig. 4a**).

Cell death induction by RSL3 causes an increase in biomarkers of lipid peroxidation in ferroptosis

Since there is no specific detection method of ferroptosis, we measured the malondialdehyde (MDA) concentration as a surrogate parameter. MDA occurs as an endproduct of ferroptotic lipid peroxidation (► **Fig. 4b**). Furthermore, the expression of ACSL4 was determined as a membrane enzyme that incorporates arachidonate lipid acids into the lipid membrane and extremely sensitive to ferroptotic lipid peroxidation [2, 3, 14]. RSL3 challenged islets showed an increased MDA concentration to 3.49 ± 0.79 nmol/mg compared to 1.63 ± 0.10 nmol/mg protein in the control group. Pretreatment with Fer-1 ($10 \mu\text{M}$) was able to attenuate RSL3 induced MDA concentrations to 2.27 ± 0.06 nmol/mg protein.

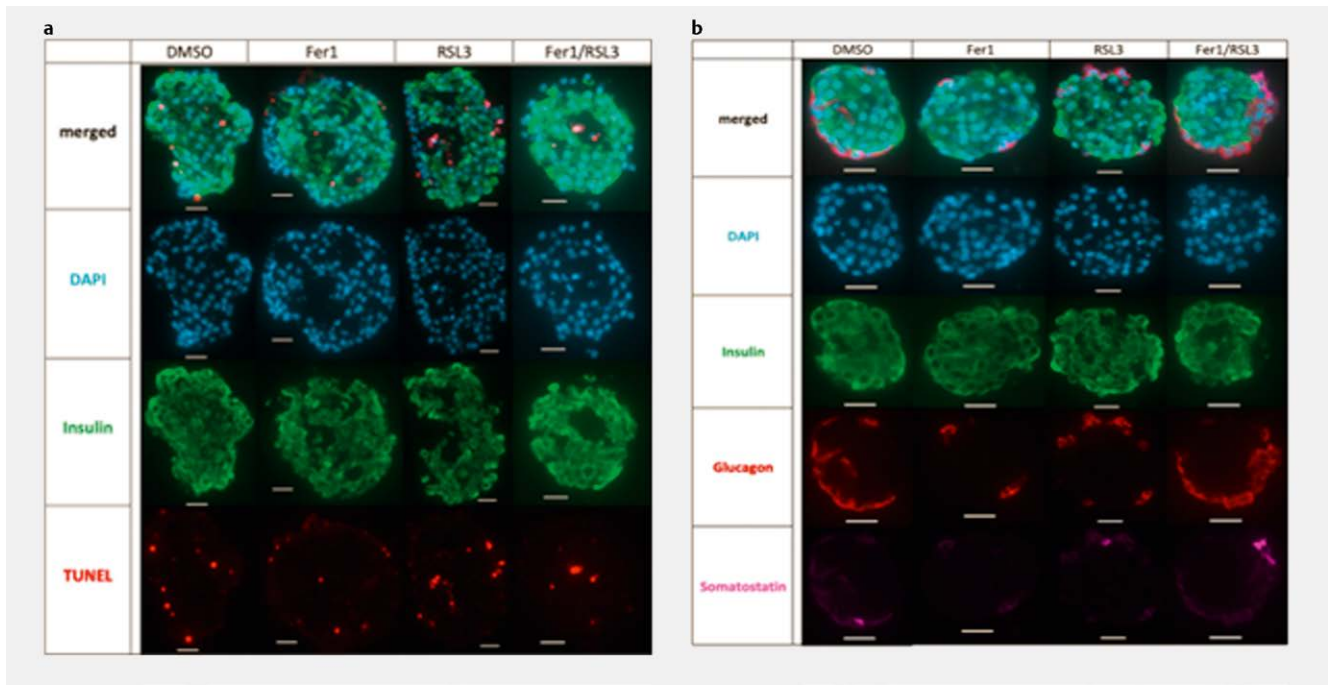
Immunostainings were performed to visualize the expression of ACSL4 in the lipid membrane of the islets (► **Fig. 6**). All imaged islets showed a positive staining for ACSL4, independently of the treatment group. However, $10 \mu\text{M}$ RSL3 enhanced ACSL4 protein level as indicated by a strong and pronounced signal compared to control group. Whereas islets treated with $10 \mu\text{M}$ Fer-1 alone or Fer-1 and RSL3 in combination led to reduced ACSL4 protein level compared to the control group.



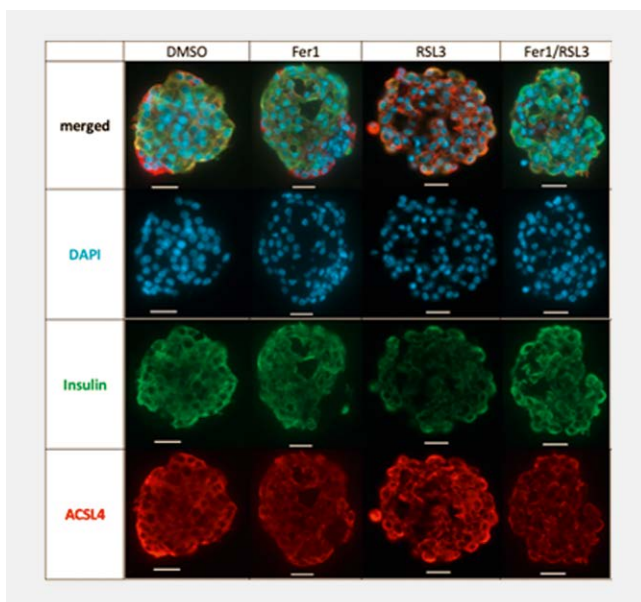
► **Fig. 4** RSL3 leads to an increase in ferroptotic metabolites: Intracellular iron (circle), zinc (square) (a) and MDA (b) concentrations were measured in nmol/mg protein of islets after treatment with RSL3 ($20 \mu\text{M}$) and Fer1 ($10 \mu\text{M}$) alone or in combination. a: Treatment with RSL3 and Fer1 led to significant changes in intracellular iron and zinc concentrations. Islets challenged with RSL3 alone showed increased iron concentrations and a significant increase in zinc concentrations compared to the control group. Incubation with Fer1 for 24 h prior to RSL3 treatment significantly reduced the effect of RSL3 alone and decreased the intracellular iron concentration. Data are presented as mean with single values from $n = 3$ single experiments, * $p < 0.05$. b: Pharmacologically induced cell death by RSL3 increased cellular stress levels as measured by MDA concentration. Treatment with RSL3 alone resulted in an increased intracellular MDA concentration of 3.49 nmol/mg compared to the control group (1.631 nmol/mg protein). The effect of RSL3 was attenuated by pre-treatment with Fer1 (2.272 nmol/mg protein). Data are presented as mean with single values from $n = 3$ single experiments.

Discussion

The aim of the study was to better understand ferroptosis mechanisms in pancreatic islets and thereby identify potential targets for protecting the islets from cell death in the context of transplantation. Minimizing and preventing cell death processes in islet transplantation to improve islet graft function has long been in the focus of islet research. The process of pancreas preparation followed by enzymatic and physically enhanced digestion of the tissue, leads to cellular stress with increasing reactive oxygen species (ROS) [15, 16]. These may induce ferroptosis at any stage of preparation



► **Fig. 5** Cell death triggered by RSL3 can be clearly distinguished from apoptosis using the TUNEL assay, as there is no increase in TUNEL positive cells due to RSL3 (a), while islet architecture remains stable after treatment with Fer1 and RSL3 (b): **a:** Immunohistochemical analysis of islets treated with 10 μ M Fer1 and 10 μ M RSL3 alone and in combination [Insulin (beta-cells, green), DAPI (core, blue), TUNEL (apoptotic DNA fragments, red)]. No difference with regard to TUNEL positive cells was detected between the different treatment groups. **b:** Immunohistochemical analysis of islets treated with 10 μ M Fer1 and 10 μ M RSL3 alone and in combination [Insulin (beta-cells, green), DAPI (core, blue), Glucagon (alpha-cells, red), Somatostatin (delta-cells, purple)]. Immunohistochemistry was performed to investigate the influence of RSL3 and Fer1 on islet architecture. Islets did not change in morphology and composition due to a treatment with Fer1 and RSL3 alone or in combination.



► **Fig. 6** Ferroptosis induction by RSL3 increases expression of the pro-ferroptotic membrane enzyme ACSL4: Immunohistochemical analysis of islets treated with 10 μ M Fer1 and 10 μ M RSL3 alone and in combination [Insulin (beta-cells, green), DAPI (core, blue), ACSL4 (membrane, red)]. Islets treated with RSL3 alone showed pronounced ACSL4 expression compared to the other groups. This suggests that treatment with RSL3 stimulates the expression of this pro-ferroptotic membrane enzyme ACSL4.

and transplantation. It has been described that apoptotic cell death peaks after five days in culture and mainly affects beta cells [17]. For ferroptosis, it is still unclear at which stage it occurs, but desferrioxamine (DFO) has been shown to improve islet and graft function. Since ferroptosis requires cellular iron, we and others have suggested that ferroptosis plays at least some role for the functional impairment of islets after isolation and transplantation [15]. Along this line, Bruni et al. first discovered that pancreatic islets were sensitive to RSL3-induced ferroptosis [6].

The current study addressed further the significance of the ferroptosis pathway and the potential of its inhibition on isolated pancreatic islets in a primary cell culture system. We focused on the cellular changes caused by RSL3 and the subsequent induction of ferroptotic cell death as well as the effects of Fer-1 on pancreatic islets. While the literature is replete on the mechanism of action of RSL3 and Fer-1 on ferroptosis for several cell types, that for pancreatic islets is just evolving [5, 18]. The current study showed that pharmaceutical induction of cell death by RSL3 causes an increase in typical ferroptosis-associated biomarkers such as iron, MDA and the membrane protein ACSL4. The findings on the features and the mechanism of ferroptosis in pancreatic islets are consistent with the observation in other cell types [1, 12, 13]. Moreover, we showed that Fer-1 attenuated the toxic consequences of RSL3 and thus protect the islets from ferroptosis. Moreover, TUNEL-staining with RSL3-challenged islets did not show an increase in apoptosis thereby clearly differentiated between apoptosis and ferroptosis cell

death processes. Our results allow for better understanding the effects of ferroptosis in islets and to differentiate more precisely between ferroptosis and other cell death mechanisms such as apoptosis. In our *in vitro* setting, islets did not show significant benefit due to the treatment with Fer-1 alone. Neither islet viability nor survival did improve due to the treatment with Fer-1 alone. Hence, islet isolation and culture *per se* seem not to substantially induce ferroptosis. However, islet function was indeed impaired due to treatment with Fer-1. As Fer-1 accumulates in the membrane of the endoplasmic reticulum (ER) one could speculate that Fer-1 is anchored in the ER membrane and thereby deteriorates islet function [19]. Anchoring Fer-1 in the ER membrane may reduce the function of the ER lipid membrane, which causes a reduction of insulin maturation and misfolded proinsulin. This could be one explanation of the reduced insulin stimulation capacity of Fer1 treated islets in contrast to control islets.

However, in the setting of intraportal islet transplantation with known drawbacks associated with hypoxic and inflammatory environmental conditions [20], it seems likely, that protective mechanisms of ferroptosis mediated through Fer-1 may have a relevant positive effect. Moreover, hepatocytes are the major iron storage in the human body, and this could further affect the impact of ferroptosis (and its inhibition) on pancreatic islet grafts [21].

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Conflict of Interest

The authors declare that they have no conflict of interest.

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