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Nanomolar resveratrol reduces early alterations of pancreatitis and pancreatic cancer in pancreatic acinar cells

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CCK: cholecystokinin
DMEM: Dulbecco's modified eagle medium
NF-κB: nuclear factor κB
PAC: pancreatic acinar cells
PI: propidium iodide
REAP: Rapid, efficient, and practical method

ABSTRACT

Resveratrol is a natural phytoalexin present in human diet with a potential beneficial effect in many diseases including chronic and acute pancreatitis, a potentially fatal disease with significant morbimortality accompanied with obscure pathogenesis and without effective treatments. Resveratrol has also been shown to be a promising anti-tumor molecule on the millimolar scale. In this study, the protective effects of nanomolar concentrations of resveratrol against early events common to pancreatic cancer and pancreatitis induced by supra-physiological concentrations of cholecystokinin (CCK, 100 nM) were evaluated. Resveratrol (200, 500 nM) reduced CCK-induced intracellular trypsin activation and cell injury in isolated rat pancreatic acinar cells (PAC). The damage induced by high CCK and the protective effect of resveratrol was also measured in pancreatic tissue explants by analyzing histological changes. Finally, the effect of resveratrol on the CCK-enhanced nuclear factor κB (NF-κB) expression was studied by Western blot experiments in the AR42J pancreatic acinar cell line. Our results show, for the first time, the ability of resveratrol to reduce premature intracellular activation of trypsin and necrosis in PAC. It is suggested that resveratrol counteracts CCK-induced pancreatic tissue breakdown by downregulation of NF-κB in the nuclear fraction. Since these effects occur at nanomolar concentrations that can be achieved by oral intake, our results suggest that dietary consumption of resveratrol could have a preventive effect and delay the evolution of pancreatitis and pancreatic cancer.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) accounts for more than 90% of all pancreatic malignancies, being one of the deadliest types of cancer (Alhobayb et al., 2021). Its incidence in the world population is estimated at $8/10^5$ person/years, with mortality of $7/10^5$ person/years (Eibl et al., 2018). Among the recognized risk factors for the development of PDAC is chronic pancreatitis, resulting from a state of persistent inflammation derived from recurrent episodes of acute pancreatitis (Alhobayb et al., 2021; Chang, 2022). In pancreatitis, inflammation of the organ is due to premature activation of pancreatic zymogens within the pancreas itself, which causes cellular autodigestion and can lead to permanent damage to pancreatic tissue (Ashraf et al., 2021).

Resveratrol (3,4′,5-trihydroxy-*trans*-stilbene; resveratrol; Fig. 1) is a naturally occurring phytoalexin present in human diet through various sources, such as wines, grapes, must, juices, peanuts, pistachios and berries (Zamora-Ros et al., 2008), that has a potential beneficial effect in many diseases (Pannu and Bhatnagar, 2019).

Resveratrol can help prevent acute pancreatitis by inhibiting the activation of inflammatory cytokines and improving microcirculation

Abbreviations: CCK, cholecystokinin; DMEM, Dulbecco's modified eagle medium; NF-κB, nuclear factor κB; PAC, pancreatic acinar cells; PI, propidium iodide; REAP, Rapid, efficient, and practical method.

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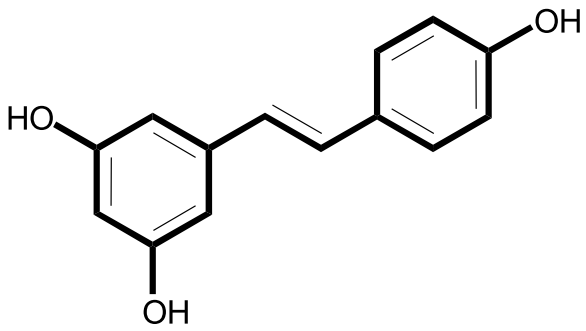

Fig. 1. Chemical structure of *trans*-resveratrol (3,4′,5-trihydroxy-*trans*-stilbene).

(Ma and Ma, 2005). It also inhibits the proinflammatory transcription factor nuclear factor κB (NF-κB) activity, enhances protective antioxidant activity, normalizes intracellular calcium management in acinar cells, and regulates several genes involved in the pathophysiology of acute pancreatitis (Agah et al., 2021). Its oral administration has been shown to reduce fibrogenesis in chronic pancreatitis by inhibiting pancreatic stellate cell activation. The inhibition of Akt and p38 mitogen-activated protein kinases signaling pathways and attenuation of retinoic acid-related orphan receptor γt (RORγt) activity could also play a role in resveratrol's beneficial effect in preventing acinar cell injury (Xia et al., 2018).

The potential effects of resveratrol on PDAC are related to less proliferation of cancer cells, with greater apoptosis and cell arrest in the presence of this agent (Joe et al., 2002). Resveratrol also decreases metastases and inhibits the appearance and viability of cancer cells, favoring their sensitivity to chemo- and radiotherapy (Xu et al. 2015). In addition, resveratrol reduced the formation of acinar to ductal metaplasia and pancreatic intraepithelial neoplasia both in vivo and in vitro, an effect related to its ability to reduce NF-κB activation (Qian et al., 2020).

Despite the beneficial effects of resveratrol on pancreatitis and PDAC, its effects on early changes common to both diseases, such as premature enzyme activation within pancreatic acinar cells (PAC) and the subsequent necrosis, have never been studied. Furthermore, the vast majority of the in vitro studies with resveratrol, including all those performed on PAC (Qian et al., 2020), have been carried out with millimolar concentrations, and only a few studies have shown specific cellular effects at concentrations at the nanomolar range (Rouse et al., 2014). This poses a problem when explaining its effects in vivo, since plasma concentrations after oral intake of resveratrol are of the millimolar order and usually ≤ 5 μM (Meng et al., 2004; Boocock et al., 2007; Wong et al., 2011). In this study we have, for the first time, examined the effects of levels of resveratrol commonly found during oral intake on various early alterations of PAC, common to pancreatitis and pancreatic cancer, generated in an experimental model of pancreatic disruption induced by supraphysiological concentrations of cholecystokinin (CCK) (Lerch and Gorelick, 2013).

Thus, hyperstimulation of freshly isolated rat PAC with CCK was used to study the effects of resveratrol on intracellular trypsin activation and cell necrosis. The effect of resveratrol on pancreatic disruption was also evaluated using histological studies of pancreatic explants hyperstimulated with CCK. We have chosen nanomolar concentrations to test whether the observed effects could explain the reported results in vivo. Finally, to investigate a possible anti-inflammatory mechanism of resveratrol action, the effect of nanomolar concentrations on the expression of NF-κB was studied by Western blot technique in the PAC rat tumor line AR42J.

2. Materials and methods

2.1. Animals and ethical approval

Male Sprague-Dawley rats (250-300 g) supplied by the University of Santiago de Compostela animal facility were used according to Orallo et al. (2000). Twenty-four hours before the experiment the rats were fasted to reduce pancreatic tissue activation on the day of its extraction. All experimental protocols were approved by the Bioethics Committee of the University of Santiago de Compostela (Spain; RD 53/2013) and the Bioethics Committee for Research (CEIC) of the Xunta de Galicia (Spain; Expedient Number: 15007/17/002), in accordance with the current Spanish and European regulations.

2.2. Evaluation of intracellular trypsin activation

PAC were isolated from the pancreas of Sprague-Dawley rats by collagenase digestion as previously described for mouse PAC (Luaces-Regueira et al., 2018).

After isolation, cells were incubated with CCK (100 nM) in the presence or in the absence of resveratrol (5, 20, 200, 500 nM), or vehicle. Then, cells were washed, suspended in assay buffer (composition in mM: Hepes 24.5, NaCl 96, KCl 6, $MgCl_2.6H_2O$ 1, $CaCl_2.2H_2O$ 0.5, NaH_2PO_4 2.5, glucose 11.5, sodium fumarate 5, sodium pyruvate 5, sodium glutamate 5; DMEM 1%; pH 7.4) and transferred to a 96-multiwell plate at different times (20, 40 and 60 min) for fluorescence measurements. An aliquot of the cells was taken before adding the treatments (time = 0 min).

Trypsin activity within PAC was measured as previously described (Luaces-Regueira et al., 2018) by a fluorometric assay using rhodamine 110 (100 μM). Measurements were performed using a fluorimeter Fluostar Optima (BMG Labtech, Germany). Enzyme activity was expressed as the increase in rhodamine fluorescence per viable cells (evaluated by propidium iodide (PI)): rhodamine fluorescence (t)/PI fluorescence (f)- PI fluorescence (t); where t is a given time and f is the end of the experiment.

2.3. Evaluation of cell injury

Cell injury was measured fluorometrically by PI intercalation into nuclear DNA as previously described (Luaces-Regueira et al., 2018). Briefly, cells treated with CCK (100 nM) in the absence or in the presence of resveratrol (5, 20, 200, 500 nM), or vehicle, were incubated with PI (1.5 mM). The level of necrosis was obtained, for all incubation times, by measuring PI fluorescence with excitation λ of 535 nm and emission λ of 617 nm, with a fluorimeter Fluostar Optima. Finally, each well was loaded with Triton X-100 (1%) to obtain the total final necrosis. Data were expressed as the percentage of necrosis at different times: % necrosis= [(final necrosis - necrosis time=t)/final necrosis)] x 100.

2.4. Culture of pancreatic tissue explants

After removing excess fatty tissue and blood vessels, the excised pancreas were placed in a 6-well plate (1 organ/well) with assay buffer containing 100 U/mL penicillin and 100 μg/mL streptomycin under sterile conditions. The plate was left in the incubator (37°C; 5% CO_2) for 30 min and then the treatments with CCK (100 nM), resveratrol (20, 200 nM), or vehicle, were added. Next, the plates were kept for another 3 h in the incubator and, subsequently, each pancreas was cut into two halves, one of which was introduced in a cassette embedded in formalin (10%) for 24 h, then in ethanol for another 24 h and, finally, in paraffin, to be sectioned and stained with hematoxylin-eosin according to standard protocols and observed under an optical microscope to obtain photomicrographs. Staining was performed by the Histology Service of the CiMUS (University of Santiago de Compostela, Spain). The other half of the pancreas was reserved for further studies.

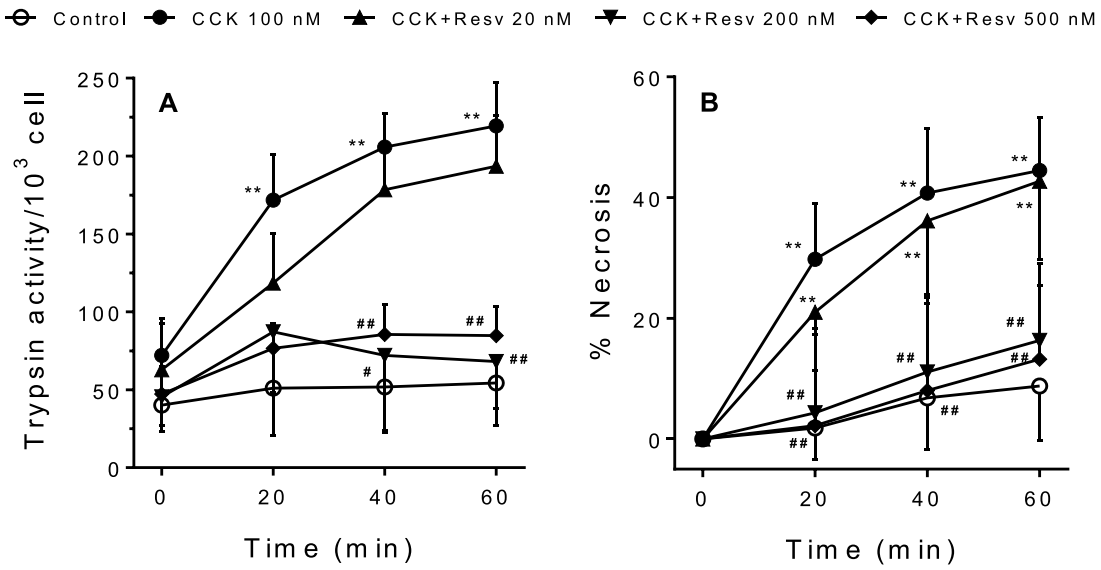

Fig. 2. Increase in intracellular trypsin activation (A) and cell necrosis (B) in rat PAC in response to CCK (100 nM) in the presence or absence of resveratrol (Resv; 20, 200, 500 nM) or vehicle (control). The results are shown as mean ± s.e.m. Level of statistical significance: $^{**}p < 0.01$ with respect to control; $\#p < 0.05$ and $\#\#p < 0.01$ with respect to CCK in the absence of resveratrol.

To evaluate the changes produced histologically in the pancreatic sections, images obtained by optical microscopy were evaluated, giving a score of 1 to 4 for each one of the established criteria to evaluate the acute necrotizing hemorrhagic pancreatitis, (Kusske et al., 1996), also used to evaluate experimentally induced acute pancreatitis (Lawinski et al., 2005).

2.5. Determination of NF-κB in AR42J cells

2.5.1. AR42J cell culture

AR42J cells (ATCC N° CRL-1492; Rockville, MD, USA), a cell line derived from a mouse pancreatic acinar carcinoma, were grown in DMEM containing L-glutamine (2 mM), penicillin (100 U/mL), streptomycin (100 μg/mL) and 10% (v/v) heat-inactivated fetal bovine serum. Cells were routinely plated in 75 cm^2 flasks, and cultured for 7-10 days (37 °C, 5% CO_2). Dexamethasone treatment dedifferentiates AR42J cells, converting them into exocrine cells (Logsdon et al., 1985). For experiments, cells were seeded at a density of ~1×10^5 cells/mL in 6-well plates and maintained at 37 °C and 5% CO_2 until confluence. Then, cells were treated with CCK (100 nM) in the absence or in the presence of resveratrol (20, 200 nM) or vehicle.

2.5.2. Protein extraction

After 48 or 72 h, protein extraction was performed using the 'REAP' (Rapid, Efficient And Practical) method to obtain the nuclear and cytosolic protein fractions as well as a complete protein sample (Suzuki et al., 2010).

The medium was removed, and the cells were washed twice with ice-cold Dulbecco's phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+}. Then, cells were scraped (1 mL of PBS/well) and collected in a conical microcentrifuge tube and then subjected to 10s spin. The supernatant was discarded, and the cell concentrate was resuspended with 1 mL PBS containing Triton (0.1%). Then, 300 μL of the cell suspension (complete protein) were removed to an ice-cold conical microcentrifuge tube. The remaining sample was subjected to another 10s spin, and 300 μL of supernatant (cytosolic protein) was collected. The rest of supernantant was discarded and the pellet was resuspended in 1mL of PBS with 0.1% Triton and resubmitted to a 10s spin, the resulting pellet being the nuclear protein. Then, 100 μL of Laemmli 4x buffer (200 mM TRIS HCl pH 6.8; 20% glycerol; 8% SDS; 20% beta-mercaptoethanol; 0.01% bromophenol blue) was added to the complete and cytosolic protein samples, and 200 μL of Laemmli 1x buffer was added to the nuclear protein samples, to prepare all of them for subsequent electrophoresis. The samples of complete and nuclear protein were sonicated twice for 5s at the 20% level of the sonicator and then all samples were boiled for 4 min.

2.5.2. Western bloth

Western blotting was used to determine protein levels of the p65 subunit of NF-κB in the cytosolic, nuclear and complete fractions of Ar42J cells. The experiments were performed as previously described (García-Morales et al., 2014). Protein concentrations were determined using bicinchoninic acid (BCA) protein assay, according to the manufacturer's protocol. After, 15 μL of each sample (30μg of protein) was separated via SDS-PAGE (8%). The gel with separated proteins was immersed in blotting buffer and transferred to a nitrocellulose membrane using an iBlot™ dry blotting system. Aiming to prevent non-specific binding, the membrane was blocked for 1h at room temperature in Blocking solution: 5% BSA in TBST (TRIS HCl 50 mM, NaCl 150 mM, pH 7.4, 0.1% Tween 20). Membranes were then incubated with rabbit anti-NF-κB primary polyclonal antibody, and with mouse anti-tubulin monoclonal antibody to confirm equivalent protein loading (both at a 1: 1000 dilution at 4°C overnight and then washed 3 times for 5 min with TBST). Next, the membranes were incubated with the horseradish peroxidase-labeled secondary antibody (10.000 dilution in 3% BSA in TBST for 2h at room temperature). After rinsing, the protein bands were visualized using the blots exposed by ECL detection reagent (EMD Millipore) followed by capture with ChemiDoc MP Imaging System (Bio-Rad Laboratories, Inc., Hercules CA, USA).

2.9. Drugs, chemicals and media

BSA, CCK, dexamethasone, eosin, formalin, hematoxylin, horseradish peroxidase conjugated secondary antibody, horseradish peroxidase-labeled secondary antibody, L-glutamine, paraffin, PBS, penicillin, PI, sodium fumarate, sodium glutamate, sodium pyruvate, streptomycin, *trans*-resveratrol, TRIS HCl, Triton X-100 and Tween-20 were from Sigma-Aldrich (Madrid, Spain). Collagenase NB 8 Broad Range was from Labclinics (Barcelona, Spain). ECL detection reagent was from EMD Millipore (Billerica, MA, USA). bis-(CBZ-L-isoleucyl-L-prolyl-L-arginine amide) dihydrochloride (BZiPAR), fetal bovine serum and rhodamine 110 were from Invitrogen (Paisley, UK). DMEM was

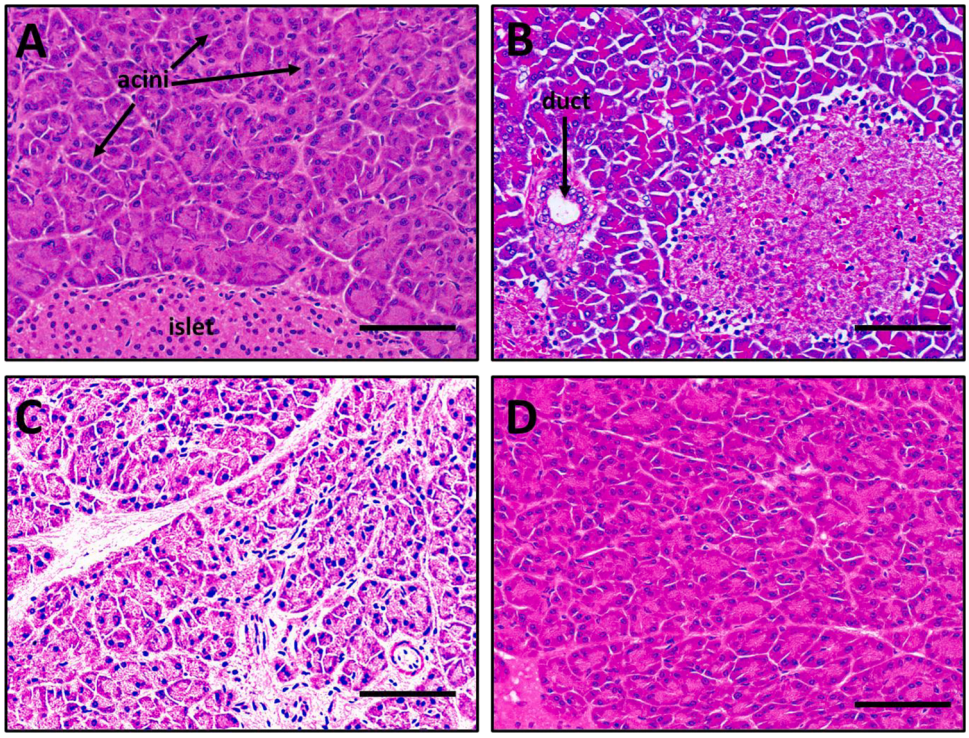

Fig. 3. Representative micrographs of histologic sections of rat pancreatic explants. (A) control, (B) CCK (100 nM), (C) resveratrol (20 nM), (D) resveratrol (200 nM). Magnification: 20x. Size bar: 100 μm.

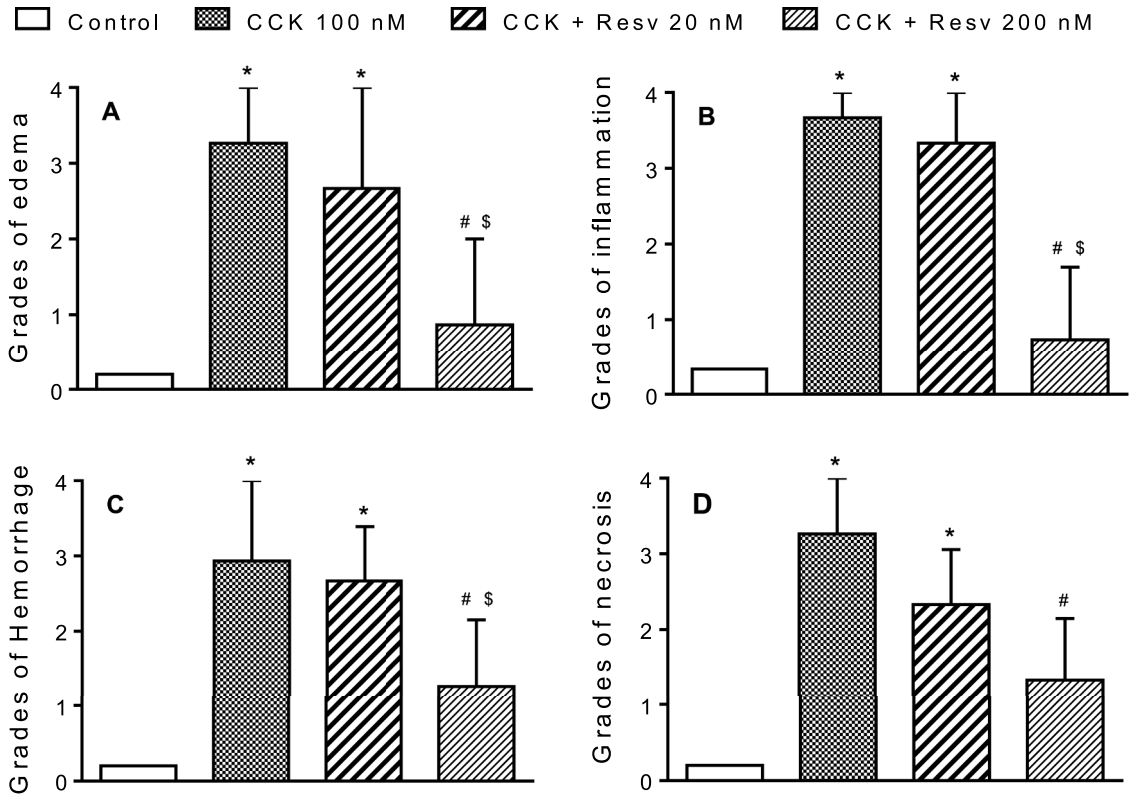

Fig. 4. Effect of resveratrol (Resv; 20, 200 nM) or vehicle (control) on the alterations induced in the pancreatic tissue by a supramaximal concentration of CCK (100 nM). The results, obtained after the histological score according to the criteria described by Kusske et al. (1996), are shown through severity of edema, inflammation, hemorrhage and necrosis (grades: 0 =none, 1 = light, 2 = mild, 3 = moderate, 4 = severe) in pancreatic tissues of respective groups: Control, CCK 100 nM, Resv 20 nM and Resv 200 nM. The bars represent the mean ± range for each group of 5 rats (measured in triplicates) using Non-parametric Kruskal-Wallis one-way ANOVA followed by Dunn's multiple comparisons test. Level of statistical significance: *$p<0.05$ vs control group; # $p<0.05$ vs CCK 100 nM group; $ $p<0.05$ vs Resv 20 nM group.

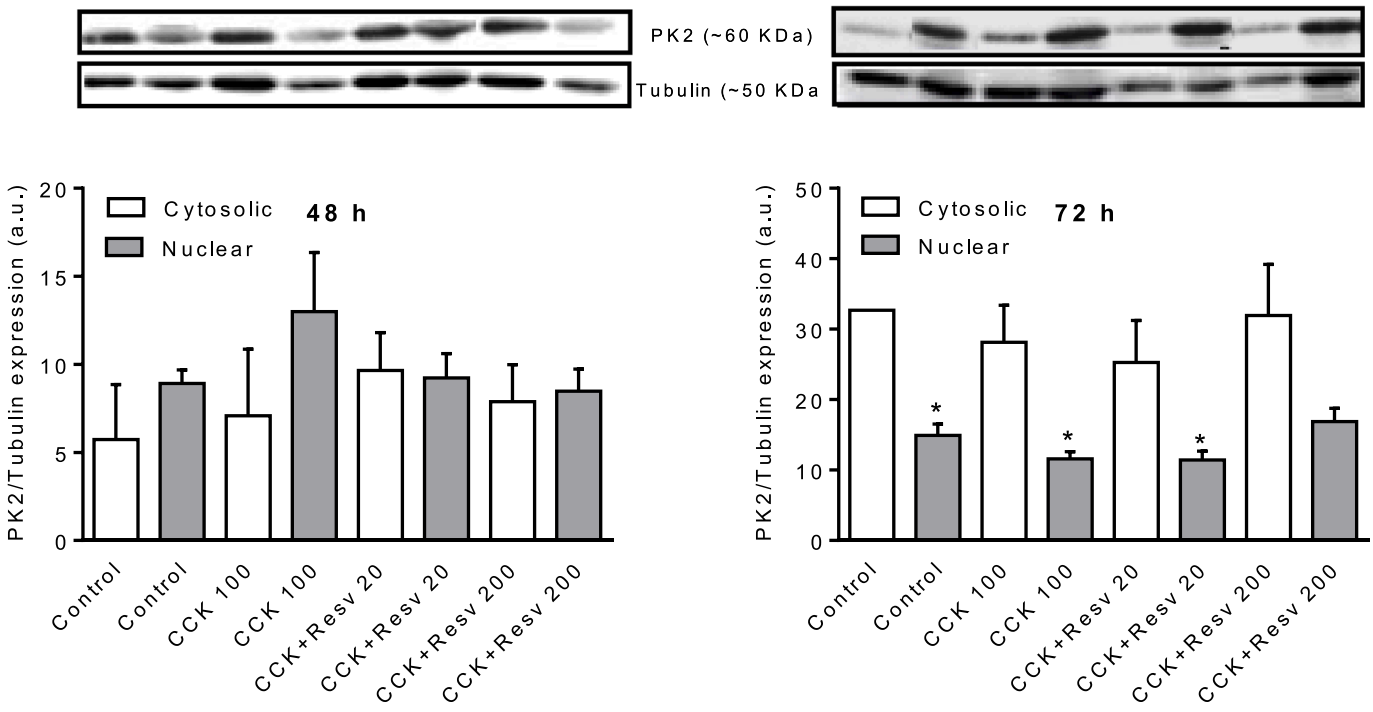

Fig. 5. Separation attempt of nuclear and cytosolic proteins by differential centrifugation in non-ionic detergent using the adapted REAP method: immunoblotting results for PK2 and tubulin expression in cytosolic and nuclear fractions of samples after 48h (A) or 72h (B) of exposition. Tubulin expression in both groups also was analyzed to confirm equivalent protein loading. (CCK 100: CCK 100 nM; CCK + Resv 20: CCK 100 nM + resveratrol 20 nM; CCK + Resv 200: CCK 100 nM + resveratrol 200 nM).

from BioWest (Nuaillé, France). Bromophenol blue bicinchoninic acid (BCA) protein assay, iBlotTM dry blotting system and rabbit anti-NF-κB p65 primary polyclonal antibody (#16758) were from Invitrogen/ ThermoFisher Scientific (Waltham, MA, USA). Mouse anti-tubulin monoclonal antibody (#EPR16774) was from Abcam (Cambridge, UK). All other chemical compounds were of analytical grade.

2.10. Data presentation and statistical analysis

Fluorescence data of trypsin activity and necrosis were obtained using Optima® software v.2.20 (BMG Labtech, Germany). A total of 10 experiments were performed with triplicate samples, only those experiments in which PAC responded significantly to the supramaximal dose of CCK were considered valid. In experiments with pancreas explants, 6 assays were performed with duplicate samples. Data are expressed as the mean ± standard error of the mean (s.e.m.). To evaluate trypsin activity and percentage of necrosis, 2-way analysis of variance (ANOVA) with the Tukey multiple comparisons test on all pairwise combinations were used. Histopathological results are presented as mean ± range, and the statistical variation was tested by Kruskal-Wallis test followed by Dunn's multiple comparisons test (Curra et al., 2013, Liu et al., 2020).

In Western blot experiments with Ar42J cells, experiments were performed with duplicate samples and all data were analyzed for protein expression using ImageJ software (see https://imagej.nih.gov/ij/docs/menus/analyze.html). In order to compare whole and cytosolic fractions, we used Student's t-test. Aiming to compare NF-κB expression between groups, we used one-way analysis of variance together with the Tukey test for comparisons of means. All the differences were considered significant at a P value of <0.05. The statistical analysis was performed by SAS Program version 9.3 (SAS Institute Inc., Gary, NC, USA) and GraphPad Prism (GraphPad Software, Inc., CA, USA).

3. Results

3.1. Resveratrol reduces CCK-induced intracellular trypsin activation and necrosis in PAC

CCK (100 nM) induced a significant activation of intracellular trypsin and necrosis in PAC at 20, 40 and 60 min that were significantly reduced by preincubation with resveratrol (200, 500 nM). As can be seen in Fig. 2, no significant differences were found in the results obtained with these two concentrations and lower concentrations of resveratrol were without effect.

3.3. Resveratrol reverses histological changes induced by CCK in pancreatic tissue

Optical microscopy shows great damage to the pancreatic tissue subjected to supraphysiological concentrations of CCK. According to the criteria described by Kusske et al. (1996), CCK increased the degree of edema, inflammation, hemorrhage and necrosis, and resveratrol (200 nM, but not 20 mM) decreased the severity of all histologic alterations (Figs. 3 and 4).

3.4. NF-κB expression

The REAP method may be used with different cell lines grown *in vitro* the advantages being the requirement for minimal time, cost and protein damage during extraction (Suzuki et al., 2010). In this study, for the first time, this technique was adapted to pancreatic cells, aiming to verify the efficiency for the extraction of total cellular proteins, cytosolic and nuclear fractions. There was a satisfactory efficiency in the extraction of total (whole) and cytosolic proteins. However, the nuclear fraction did not present the necessary purity for investigating the NF-κB. This was proven through pyruvate kinase and tubulin expression (classical cytoplasmic biomarkers) which were also clearly detected in nuclear

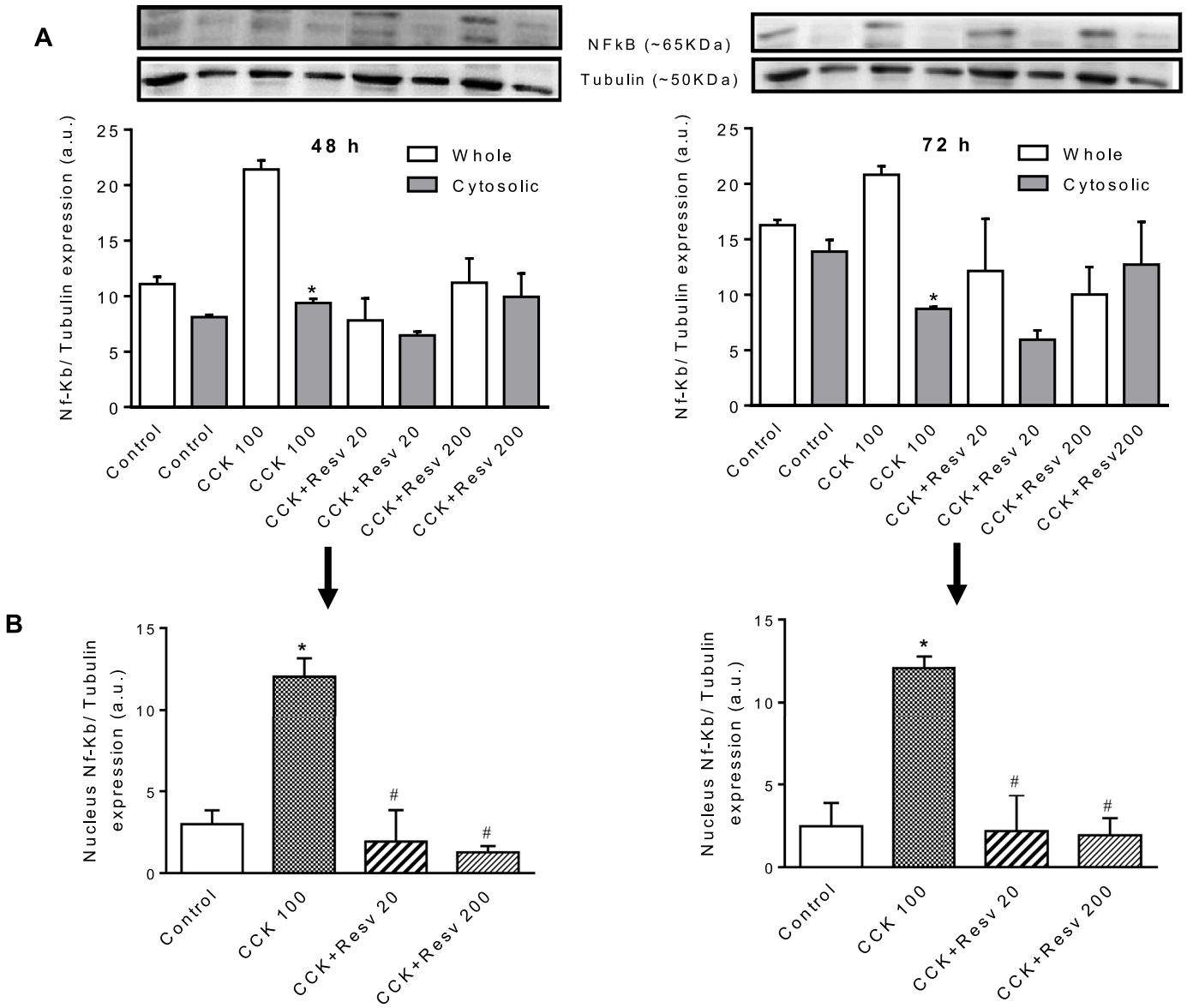

Figure 6. Effects of 48 and 72 h of treatment with resveratrol 20 nM (Resv 20) or 200 nM (Resv 200) on the CCK (100 μM)-induced increase of the expression of the p-65 subunit of NF-κB in the (A) whole and cytosolic fractions and (B) nuclear fractions of AR42J cells evaluated indirectly from the subtraction of expression between the samples containing the total cells (whole cell) and the cytosolic fraction. The protein expression was normalized with respect to the expression values of tubulin and the results are shown as mean ± s.e.m (n=3). Level of statistical significance: *p <0.05 with respect to respective whole group (A) or control group (B). #p <0.05 with respect to respective CCK 100 group.

fractions (Fig. 5). As an alternative, the expression of nuclear NF-κB was deduced by the subtraction of expression between the samples containing the total cells (whole cell) and the cytosolic fraction. Interestingly, after this analysis 'whole minus cytosolic fraction', the expression of nuclear NF-κB (either 48 or 72h) was higher in the CCK-treated group (~2.5-fold, p <0.05) and resveratrol prevented this difference in both groups (48 and 72h) (Fig. 6).

4. Discussion

This study demonstrates, for the first time, the ability of resveratrol to prevent premature activation of trypsin within PACs and subsequent necrosis, two pathognomonic events common to the initial stages of pancreatitis and pancreatic cancer. In addition, these effects were caused by nanomolar concentrations of resveratrol, which would help explain the previously described beneficial pancreatic effects after oral intake of resveratrol, which generates plasma concentrations of that order (Wong et al., 2011). Contrary to our results, the *in vivo* treatment with resveratrol did not inhibit trypsin activity or prevent taurocholate-induced parenchymal necrosis in rat pancreas (Gulcubuk et al., 2014).

In accordance with the results obtained in PAC, the photomicrographs of pancreatic explants show much structural damage in the tissues exposed to a high concentration of CCK, which was reduced in the presence of nanomolar resveratrol. These results are consistent with previous histological evaluations showing that resveratrol significantly reduced pancreatic tissue edema, acinar vacuolization and total histological damage in CCK-induced acute edematous pancreatitis (Szabolcs et al., 2006). Similarly, resveratrol protected the ultrastructure of rat pancreatic tissue from the damage caused by tert-butyl hydroperoxide-induced acute pancreatitis (Lawinski et al., 2005) and partially prevented leukocyte infiltration, a parameter that contributes to the course of acute pancreatitis (Gulcubuk et al., 2014). It should be noted that these previous results were obtained after in vivo treatment with

resveratrol.

The signaling pathway of NF-κB is critical in the pathogenesis of acute pancreatitis, by mediating the expression of genes involved in inflammation and the immune response (Kim et al., 2017) and plays a crucial role in pancreatic cancer development and progression (Pramanik et al., 2018). The results of the present study show an inhibition of NF-κB using nanomolar concentrations of resveratrol. This effect had only been previously described in endothelial cells (Pellegatta et al., 2003). In contrast, the ability of higher (millimolar) concentrations of resveratrol to reduce NF-kB signaling has been previously described in numerous preparations, including macrophages (Tsai et al., 1999), human histiocytic lymphoma, human epithelial cells, glioma cells, T cells (Manna et al., 2000) and mouse pancreatic acinar cells (Qian et al., 2020). On the contrary, an inhibition of NF-κB activation could not be demonstrated in CCK-induced experimental pancreatitis (Szabolcs et al., 2006).

Thus, the results obtained in this study are the first to show an anti-inflammatory activity of nanomolar resveratrol in PAC by inhibition of NF-κB signaling, which correlates well with similar results obtained after oral administration of resveratrol in rodents (Ginés et al., 2017; Darwish et al., 2021).

The REAP protocol, adapted for the first time to pancreatic cells in this work, is faster and cheaper than the conventional one. However, our data show that, for pancreatic cultures, it is useful for the extraction of the total protein sample, but it is not as sensitive to separate cytosolic and nuclear fractions as observed in cultures of neurons (Suzuki et al., 2010). Due to their fragile membrane and consequent limited nuclear detergent stability, PAC show a great challenge for standard nuclear isolation protocols (Poglitsch et al., 2011). This evidence is justified by the presence of tubulin and PK2 in the nuclear samples. However, observing the expression of the whole and cytosolic fractions, the greatest difference of expression between them appears in the CCK-treated group demonstrating, even if indirectly, that nuclear expression of NF-κB was reduced by nanomolar resveratrol.

It is important to note that, in our experiments, significant results were obtained with resveratrol concentrations in the nanomolar range. Although a few studies have shown significant in vitro effects of resveratrol in the nanomolar range, most of studies with resveratrol in vitro have used higher concentrations, in the micromolar range, including all previous studies in pancreatic tissue (Xia et al., 2018).

In conclusion, we have here demonstrated, for the first time, that resveratrol decreases premature intracellular activation of trypsin and necrosis in PAC, two pathophysiological events common to the initial stages of pancreatitis and pancreatic cancer. It also counteracts CCK-induced pancreatic tissue breakdown, perhaps by downregulation of nuclear NF-κB. The fact that these effects occur at nanomolar concentrations, achievable in human plasma by oral intake of resveratrol, is an explanation for various beneficial effects at the pancreatic level found in previous *in vivo* studies.

Assuming that resveratrol could exhibit similar behavior in humans *in vivo*, its dietary intake could be protective during the initial stages of pancreatic diseases and delay the evolution of these diseases. This action would be of significant benefit in helping to prevent or delay the onset of these diseases, as well as slowing down their progression. In addition, resveratrol could serve as a structural model for the design of new molecules with potential benefits against pancreatic diseases.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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