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Unsaturated but not saturated fatty acids induce transcriptional regulation of CCL2 in pancreatic acini. A potential role in acute pancreatitis

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ABSTRACT

Fatty acids (FAs) are massively released from peripancreatic fat during acute pancreatitis (AP) and they were shown, as a whole, to induce inflammatory response in pancreatic acini. We investigated the mechanisms triggered by the major saturated FAs (SFAs) and unsaturated FAs (UFAs) in modulating the expression of chemokine (C–C motif) ligand 2 (CCL2) in acinar cells. Pancreatic acini of control rats were treated with palmitic acid (PA) or stearic acid (SA), as SFAs, or oleic acid (OA) or linoleic acid (LA), as UFAs. By using specific inhibitors, the involvement of MAPKs (JNK, ERK, p38), JAK, NF-κB and STAT3 pathways was assessed. The role of PPARγ pathway was studied by using 15-Deoxy-Δ(12,14)-prostaglandin J(2) (15d-PGJ2). CCL2 mRNA was analyzed by qRT-PCR. By western blot, phosphorylated forms of MAPKs and JAK as well as IκB-α were analyzed in cytoplasm and p65-NF-κB and phospho-STAT3 in nucleus. No effect was found in PA- or SA-treated acini. Conversely, in response to OA or LA, MAPKs and JAK acted as upstream signals, driving the CCL2 up-regulation transcriptionally mediated by the synergic action of NF-κB and STAT3. By blocking the activation of NF-κB and STAT3, 15d-PGJ2 totally inhibited the OA- and LA-induced CCL2 overexpression. We conclude that the most common UFAs, but not the SFAs, represented in peripancreatic fat and released during AP, are capable of up-regulating the acinar expression of CCL2, which depends on the activation of MAPK/JAK-mediated NF-κB and STAT3 pathways. By targeting both transcription factors, PPARγ agonists could be indicated as potential therapy in AP.

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1. Introduction

Elevated concentration of free fatty acids (FAs) in plasma is a common feature in inflammatory diseases, including acute pancreatitis (AP) [1,2]. Regardless of AP etiology, digestive enzymes, such as lipases and phospholipase A2, are leaked out from the injured acinar cells into the pancreatic interstitium and blood stream [3]. As a result of the lipase action on peripancreatic adipose tissue, high amounts of FAs are generated, an event associated with multisystem organ failure during severe forms of AP [4,5] which is especially relevant in obesity. With regard to this, obesity is considered a risk factor that aggravates the progression of AP in humans [5–7] and animal models [8,9], although the underlying mechanisms are not fully understood.

Noxious effects have been attributed to FAs due to their cytotoxic action disrupting cell membranes [10], as well as to their role in modulating inflammation [11]. Currently, there is growing interest in investigating the involvement of these lipid derivatives in diverse inflammatory disorders; however, information about the cellular mechanisms associated with the FA response is incomplete. Contradictory effects have been reported for individual FAs on different cell types. Thus, pro-inflammatory [12] and no effects [13] have been found in response to saturated FAs (SFAs). Regarding unsaturated FAs (UFAs), anti-inflammatory [14], pro-inflammatory [13,15,16] and no effects [17] have also been reported. Controversial data may be explained because the response might be cell dependent and specific for each FA according to its chemical structure. So, it is important to assess the effects exerted by individual UFAs and SFAs on the same cell type as well as the underlying mechanisms to trigger the cell response.

The effects of different lipid components on pancreatic acini have not yet been thoroughly investigated despite the relevance they may have in the pathophysiology of AP. In a recent study [18], we reported that total lipids as well as the FA fraction obtained from necrotic adipose tissue of rats with severe AP up-regulated the expression of inflammatory mediators in pancreatic acini. Given that in peripancreatic fat necrosis of rats with AP, the most abundant SFAs proved to be palmitic acid (PA, C16:0) and stearic acid (SA, C18:0), and the major UFAs

Abbreviations: AP, acute pancreatitis; CCL2, chemokine (C–C motif) ligand 2; ERK, extracellular signal-regulated kinase; FA, fatty acid; JAK, Janus kinase; JNK, Jun NH2-terminal kinase; LA, linoleic acid; MAPKs, mitogen-activated protein kinases; NF-κB, nuclear factor-kappa B; OA, oleic acid; PA, palmitic acid; PPAR, peroxisome proliferator-activated receptor; SA, stearic acid; SFA, saturated fatty acid; STAT, signal transducer and activator of transcription; UFA, unsaturated fatty acid; 15d-PGJ2, 15-Deoxy-Δ(12,14)-prostaglandin J2

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were oleic acid (OA, C18:1) and linoleic acid (LA, C18:2) [18], the aim of the current study was to assess their individual role in triggering mechanisms that lead to the inflammatory response in acinar cells. As a representative inflammatory mediator with a key role in AP, we analyzed the expression of chemokine (C–C motif) ligand 2 (CCL2) in pancreatic acini exposed to the above-mentioned FAs and the underlying signaling pathways involved in the FA response. We show that the most abundant UFAs, but not the SFAs, in necrotic peripancreatic fat tissue of rats with AP activated molecular pathways which lead to CCL2 overexpression in acinar cells. The relevance of this study lies in furthering the understanding of the role of FAs in the pathophysiology of AP and in the approach of potential therapeutic strategies to the disease.

2. Materials and methods

2.1. Chemicals

Collagenase type XI, N-(2-hydroxyethyl) piperazine-N′-(2-ethanesulfonic acid) (HEPES), amino acids solution, bovine serum albumin (BSA), palmitic acid, stearic acid, oleic acid, linoleic acid, soybean trypsin inhibitor (STI), phenylmethanesulfonyl fluoride (PMSF), dithiothreitol (DTT), digitonin, protease inhibitor cocktail, phosphatase inhibitor cocktail, Nonidet P-40, SP600125, AG-490 and 15-Deoxy-Δ(12,14)-prostaglandin J(2) (15d-PGJ2) were supplied by Sigma Chemical Co. (Madrid, Spain). SB202190 was from Calbiochem (Darmstadt, Germany). GDC-0994, SH-4-54 and JSH-23 were kindly donated by Selleckchem (Munich, Germany). Other standard analytical grade laboratory reagents were obtained from Merck (Darmstadt, Germany).

2.2. Animals

Male Wistar rats (250–300 g body weight) were supplied by the experimental animal service of the University of Salamanca (Spain). They were housed individually in cages, maintained at 22 ± 1 °C using a 12 h light/dark cycle and fed with standard laboratory chow (Teklad-Harlan 2014, Mucedola, Milan, Italy) and tap water *ad libitum*. The animals were fasted overnight before the experiments but they were allowed free access to water. The study was performed in accordance with European Community guidelines (2010/63/EU) and approved by the Institutional Animal Care and Use Committee of the University of Salamanca (Spain).

2.3. Isolation and treatment of pancreatic acini

After 12 h fasting and under anesthesia with sodium pentobarbital (3 mg/100 g body weight, intraperitoneally), pancreas of control rats was removed in order to isolate pancreatic acini by digestion with collagenase as previously described [18]. Afterwards, pancreatic acini were resuspended in Na-HEPES solution (pH 7.4) enriched with 14 mM glucose and 1% essential aminoacid mixture and containing 1% (*w*/*v*) BSA, 0.01% (*w*/*v*) STI, and 0.5 mM $CaCl_2$ and incubated (5% CO_2, at 37 °C) for 1 h in the presence of the following FAs: PA (C16:0), SA (C18:0), OA (C18:1) and LA (C18:2) or the vehicle (EtOH) at concentrations ≤1% (controls). In some experiments, acini were pre-treated with SP600125 (50 μM), GDC-0994 (50 μM) or SB202190 (50 μM), as inhibitors of JNK, ERK and p38-MAPK, respectively; AG-490 (50 μM) as a JAK inhibitor; JSH-23 (30 μM), SH-4-54 (10 μM), as inhibitors of NF-κB and STAT3, respectively; or the vehicle dimethyl sulfoxide (DMSO) at concentrations ≤0.3%. In another set of experiments, the effect of PPARγ activation on the FA response was evaluated by incubating acinar cells with 15d-PGJ2 (10 μM), a specific PPARγ agonist, added 5 min after initiating the incubation in the absence or presence of each FA.

2.4. Lactate dehydrogenase (LDH) activity

LDH leakage was evaluated as a measurement of the cell damage. LDH activity was analyzed according to the method of Gutmann and Wahlefel [19]. Cellular LDH was measured after lysis of pancreatic acini with a 50 mM Tris–HCl buffer (pH 7.2) containing 50 mM β-glycerophosphate, 15 mM $MgCl_2$, 15 mM EDTA, 10 mM PMSF, 1 mM DTT and 150 μg/ml digitonin. The released LDH was measured in the incubation medium. Changes in absorbance due to β-nicotinamide dinucleotide$^+$ formation were recorded at 339 nm at 30 °C. We computed the total LDH and calculated the LDH leakage as percentage of the total measured inside and outside cells.

2.5. Western blot analysis of MAPKs, JAK, NF-κB and STAT3 activation

Cytoplasmic and nuclear extracts of pancreatic acini were obtained as previously reported [18]. Samples (30 μg) were individually separated by 12% SDS-PAGE and electrophoretically transferred to a nitrocellulose membrane. Phospho-MAPKs (p-MAPKs), phospho-JNK (p-JAK) and IκB-α were analyzed in cytoplasm and p65 and phospho-STAT3 (p-STAT3) in nuclear extracts. Non-specific binding was blocked by incubating the blot in Tris-buffered saline (TBS) pH 7.6, containing 0.1% (*v*/*v*) Tween 20 and 5% (*w*/*v*) nonfat dry milk for 1 h. Afterwards, blots were incubated with the primary antibody against either each of the three p-MAPKs (p-JNK, p-ERK, p-p38), p-JAK, IκB-α, p65 or p-STAT3 (Cell Signaling Technology, Beverly, MA) at 1:1000 dilution in TBS buffer pH 7.6, containing 0.1% (*v*/*v*) Tween 20 and 5% (*w*/*v*) BSA overnight at 4 °C. α-Tubulin and lamin B1 (Cell Signaling Technology, Beverly, MA) were used as the load control of cytoplasmic and nuclear proteins, respectively. After washing with TBS containing 0.1% Tween 20, the blots were incubated for 1 h at room temperature with the respective horseradish peroxidase-conjugated secondary antibody at 1:2000 dilution in TBS buffer pH 7.6, containing 0.1% Tween 20 and 5% (*w*/*v*) nonfat dry milk and finally they were developed for visualization. The bands were detected with the Phototope-HRP Detection kit (Cell Signaling Technology, Beverly, MA). Image J 1.32 software from http://rsbweb.nih.gov/ij/download.html was used to quantify the intensity of the bands. Relative protein levels were calculated compared to either the α-tubulin or lamin B1 standards. Results are expressed as fold increase *vs* control acini.

2.6. Analysis of CCL2 mRNA by quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was isolated from pancreatic acini using the RNAeasy kit treated with amplification grade DNase 1 (Qiagen, Valencia, Spain) according to the manufacturer's instructions. The integrity of RNA based on the presence of well-defined 28S and 18S rRNA bands was assessed in 1% agarose gel and RNA concentration was measured by spectrometry.

For first strand cDNA synthesis, 1 μg of total RNA was reverse-transcribed using the iScript cDNA synthesis kit for RT-qPCR (Bio-Rad Laboratories, Hercules, CA). cDNA was amplified by a polymerase chain reaction (PCR) on a StepOne system (Applied Biosystems, CA, USA) using the IQ SYBR® Green Supermix (Bio-Rad Laboratories) and specific primers (Roche) for each gene: chemokine (C–C motif) ligand 2 (CCL2) (NM_031530) forward: 5′-TAGCATCCACGTGCTGTCTC-3′ reverse: 5′-CCGACTCATTGGGATCATCT-3′ and 18S (NM_046237) forward: 5′-AGTCCCTGCCCTTTGTACACA-3′ and reverse: 5′-GATCCGAG GGCCTCACTAAAC-3′. The cDNA was amplified by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 20 s. Amplifications were performed in triplicate and the melting curves were analyzed to validate product specificity. The ratio of the relative expression of target genes to 18S (internal standard) was calculated by using the ΔΔCt method. Results were expressed as fold increase over untreated cells.

2.7. Statistical analysis

Results are expressed as means ± SEM and analyzed by one-way ANOVA followed by a Tukey post-hoc test for multiple comparisons. Two-way ANOVA was applied in experiments in which two variables (dose and treatment) were used. p values lower than 0.05 were considered to be statistically significant.

3. Results

3.1. Dose–response of LDH leakage and CCL2 mRNA expression

The measurement of LDH leakage (Fig. 1A) revealed no significant cell injury in pancreatic acini incubated with PA or SA at concentrations ≤1200 μM in comparison with control acini. A rise in LDH leakage was found in pancreatic acini exposed to increasing concentrations of OA or LA, reaching statistical ($p < 0.01$) significance at 600 μM and 1200 μM. Significant differences were also found between the SFAs (PA, SA) and UFAs (OA, LA) at 600 μM ($p < 0.05$) and 1200 μM ($p < 0.01$).

The expression of acinar CCL2 mRNA in response to FAs is shown in Fig. 1B. No significant change in the acinar expression of CCL2 was found in response to either PA or SA in a range of concentrations of 0–1200 μM. Conversely, as regards UFAs, CCL2 mRNA expression increased in response to OA or LA at 300 μM, but it declined at lethal concentrations. Significant ($p < 0.01$) differences were also found between the SFAs (PA, SA) and UFAs (OA, LA) at 300 μM.

No significant effect was found in acini exposed to the vehicle.

3.2. Effects of fatty acids on the activation of MAPKs, JAK, NF-κB and STAT3

Activation of MAPKs (JNK, ERK and p38) and JAK was analyzed by measuring their phosphorylated forms by western blot in cytosolic extracts (Fig. 2). PA and SA did not exert significant effects on either MAPK or JAK activation. By contrast, significant increases in p-JNK (p54, p46), p-ERK (p44, p42), p-p38 and p-JAK were found in pancreatic acini exposed to either OA or LA.

Fig. 3 depicts data of the activation of NF-κB (cytosolic IκB-α and nuclear p65) and STAT3 transcription factors. No significant effect was exerted by PA or SA on NF-κB or STAT3 activation. In contrast, OA and LA significantly ($p < 0.01$) reduced the cytosolic levels of IκB-α and significantly increased p65 at the nuclear level. Regarding STAT3 activation, OA and LA significantly ($p < 0.01$) increased the nuclear p-STAT3 levels.

No change was found in response to the vehicle.

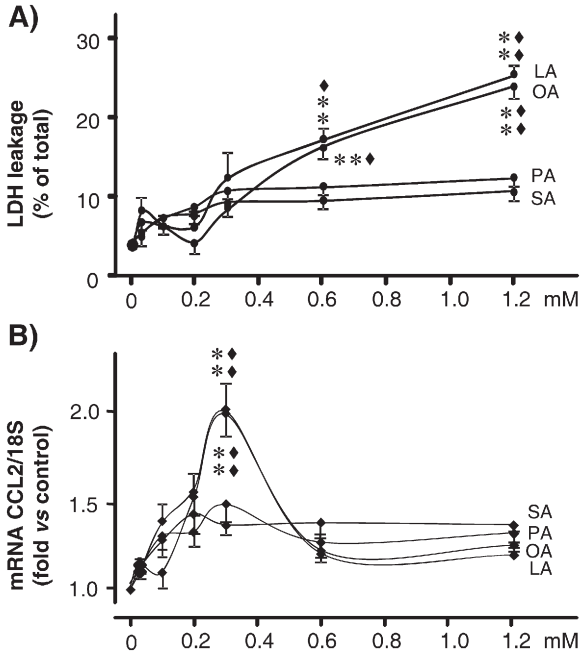


Fig. 1. Unsaturated fatty acids (UFAs), but not saturated fatty acids (SFAs), induced cell injury and CCL2 up-regulation. Pancreatic acini were incubated for 1 h in the presence of the following FAs (0–1200 μM): palmitic acid (PA), stearic acid (SA), oleic acid (OA), linoleic acid (LA) or EtOH, as the vehicle, (controls). A) Cell injury was measured by leakage of LDH (% of the total LDH measured inside and outside cells). B) mRNA expression of CCL2 was analyzed by qRT-PCR with 18S as internal standard. Values are means ± SEM of five experiments. Two-way ANOVA showed significant differences *vs* control acini when the effect of concentration was evaluated (**$p < 0.01$) and *vs* PA and SA (♦$p < 0.05$, ♦♦$p < 0.01$) when, at each dose, the effect of the different FAs was analyzed.

3.3. CCL2 mRNA up-regulation is transcriptionally regulated by NF-κB and STAT3

As shown in Fig. 4, the acinar CCL2 mRNA up-regulation induced by OA or LA was significantly ($p < 0.01$) reduced by pre-treatment with the NF-κB inhibitor JSH-23 as well as with the STAT3 inhibitor SH-4-54. Nevertheless, the values obtained in acini pre-treated with the inhibitors were still found to be significantly ($p < 0.01$) higher than in untreated acini.

3.4. Involvement of MAPKs and JAK in the NF-κB- and STAT3-up-regulated CCL2 mRNA expression

Fig. 5 shows the results obtained by using specific inhibitors to verify the role of MAPKs and JAK on the activation of NF-κB and STAT3 as well as on CCL2 mRNA up-regulation mediated by OA or LA. The densitometric analysis of IκB-α (Fig. 5A) and p65 (Fig. 5B) revealed that SP600125, GDC-0994 or SB202190, inhibitors of JNK-, ERK- and p38-MAPK, respectively, as well as AG-490, a JAK inhibitor, significantly ($p < 0.01$) reduced the OA- or LA-mediated activation of NF-κB. The IκB-α degradation induced by either OA or LA was significantly ($p < 0.01$) attenuated by inhibition of either JNK, ERK, p38 or JAK. Reciprocally, the increased p65 levels found in the nucleus of pancreatic acini treated with either OA or LA were significantly ($p < 0.01$) reduced by either each MAPK or JAK. Similarly, OA- or LA-induced STAT3 activation (Fig. 5C) was significantly ($p < 0.01$) reduced in the presence of SP600125, GDC-0994, SB202190 or AG-490. In the same line, pre-treatment with inhibitors of either MAPKs or JAK also significantly ($p < 0.01$) decreased the CCL2 mRNA up-regulation mediated by OA or LA (Fig. 5D).

Despite the presence of the inhibitors the nuclear levels of p65 and STAT3 as well as the CCL2 mRNA expression remained at values significantly higher than in untreated acini.

3.5. Effect of PPARγ activation on the inflammatory acinar response

As shown in Fig. 6, the presence of the PPARγ agonist 15d-PGJ2, in the incubation medium significantly reduced the activation of JNK-, ERK- and p38-MAPK induced by either OA or LA to values not significantly different from those of control acini. The PPARγ agonist also repressed the JAK activation mediated by OA or LA. No effect was exerted by 15d-PGJ2 on control acini.

As can be seen in Fig. 7A and B, 15d-PGJ2 blocked the OA- or LA-mediated NF-κB activation, by reducing the cytosolic IκB-α degradation and p65 at the nuclear level. The PPARγ agonist also inhibited the STAT3 activation induced by OA or LA (Fig. 7C). Regarding CCL2 mRNA expression (Fig. 7D), 15d-PGJ2 repressed the CCL2 up-regulation induced by OA or LA.

4. Discussion

It is increasingly accepted that FAs play a pivotal role in modulating inflammation, but the understanding of the mechanisms involved in this response remains unclear. Given that they are released in high amounts from peripancreatic adipose tissue during AP, the assessment

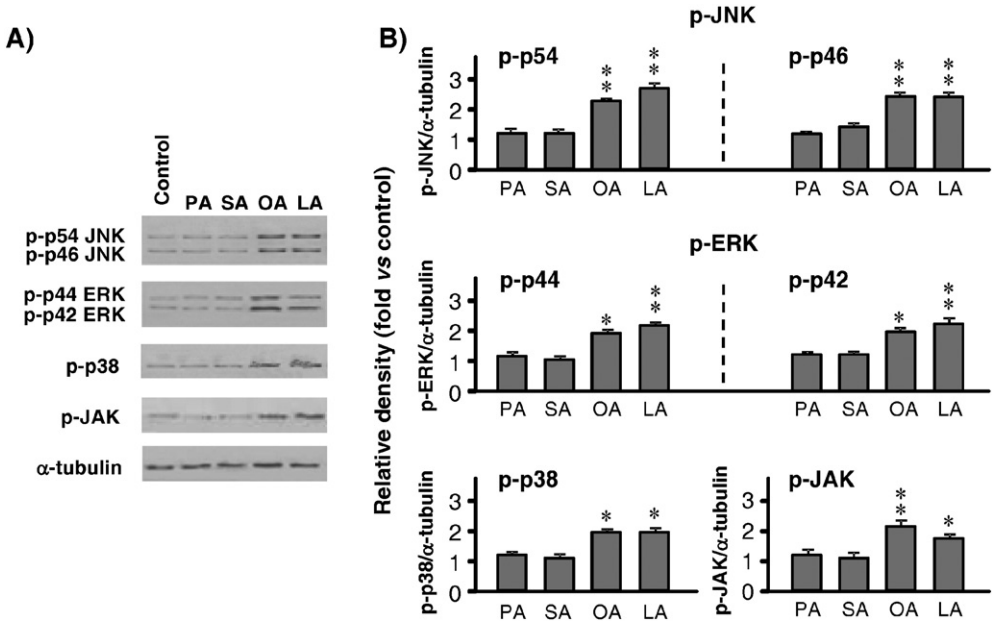


Fig. 2. Unsaturated fatty acids (UFAs), but not saturated fatty acids (SFAs), activated MAPKs and JAK. Pancreatic acini were incubated for 1 h in the presence of the following FAs (300 μM): palmitic acid (PA), stearic acid (SA), oleic acid (OA), linoleic acid (LA) or EtOH, as the vehicle, (controls). Phosphorylated MAPKs (p-MAPKs): c-Jun NH2-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), p38 and phosphorylated Janus kinase (p-JAK) were analyzed in cytoplasmic extracts by western blot. Representative blots of five experiments (A) and densitometric analysis of the band intensity (B) are shown. Densities (arbitrary units) were normalized with those of α-tubulin (loading control). Results (means ± SEM) are expressed as fold increase *vs* control acini. $^{*}p < 0.05$, $^{**}p < 0.01$ as compared with control acini.

of the FA effects on acinar cells, the initial source of inflammatory mediators during the disease [20], would give new insights into the pathophysiology of AP. This proposal would be especially relevant in the context of obesity given that FAs are massively released from peripancreatic fat tissue during AP. Furthermore, obese subjects develop severe forms of the disease with abundant peripancreatic necrosis [5–9] which have proven to be enriched in UFAs [5].

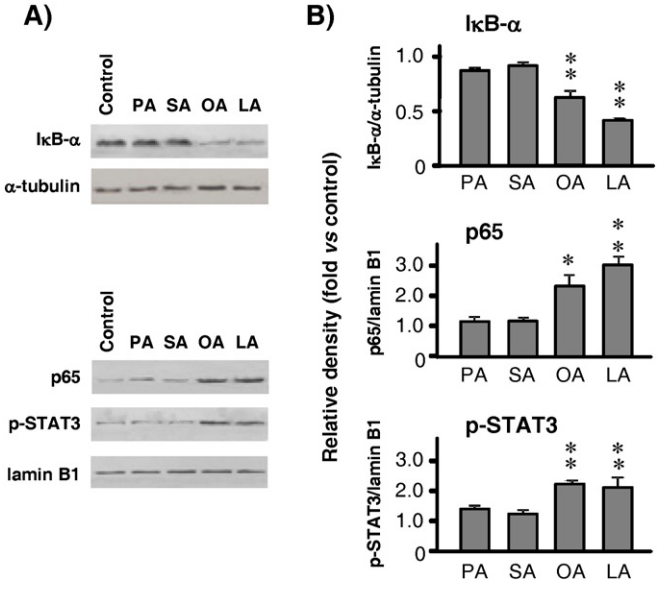


Fig. 3. Unsaturated fatty acids (UFAs), but not saturated fatty acids (SFAs), activated NF-κB and STAT3. Pancreatic acini were incubated for 1 h in the presence of the following FAs (300 μM): palmitic acid (PA), stearic acid (SA), oleic acid (OA), linoleic acid (LA) or EtOH, as the vehicle (controls). Western blots of cytosolic IκB-α (top), nuclear p65 (middle) and nuclear phosphorylated STAT3 (p-STAT3) (bottom) are shown. Representative blots of five experiments (A) and densitometric analysis of the band intensity (B) are shown. Densities (arbitrary units) were normalized with those of α-tubulin or lamin B1 (loading control for cytosolic or nuclear proteins, respectively). Results (means ± SEM) are expressed as fold change *vs* control acini. $^{*}p < 0.05$, $^{**}p < 0.01$ as compared with control acini.

Since both pro- and anti-inflammatory effects have been reported for FAs [13,15,17], knowledge of the individual effects of the major FAs in the fat tissue is of great interest considering that the specific action of each FA could determine the fate of this inflammatory disease. The use of the SFAs (PA, SA) with different number of carbon atoms and the UFAs (OA, LA) with the same carbon but different number of double bonds will allow us to ascertain whether the acinar response depends on the FA chemical structure and, in such a case, elucidate which the key molecular feature is.

In order to mechanistically explain the effects of UFAs and SFAs, we analyzed the main intracellular signaling pathways involved in the inflammatory response in acinar cells. Our data revealed that JNK-, ERK-, and p38-MAPKs as well as JAK played a key role as upstreams in the events which led to the CCL2 up-regulation mediated by the UFAs OA and LA. It is widely accepted that the phosphorylation required for the transcriptional activation of NF-κB is MAPK-dependent, while the activation of STAT3 transcription factor is generally associated with JAK

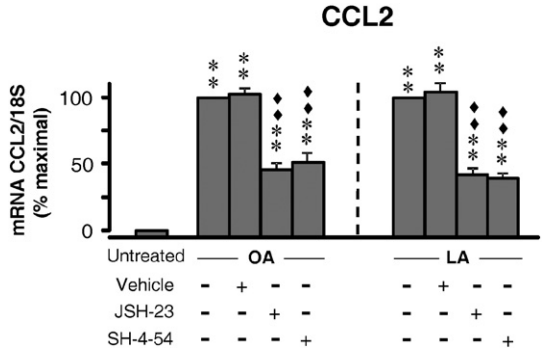


Fig. 4. CCL2 up-regulation induced by unsaturated fatty acids is dependent on NF-κB and STAT3. Pancreatic acini exposed for 1 h to 300 μM of oleic acid (OA) or linoleic acid (LA) were pre-treated for 45 min with the vehicle (DMSO), the NF-κB inhibitor JSH-23 (30 μM) or the STAT3 inhibitor SH-4-54 (10 μM). CCL2 mRNA expression was analyzed by qRT-PCR with 18S as internal standard. Results (means ± SEM) from five experiments are expressed as percentages of the maximal response. $^{**}p < 0.01$ as compared with untreated acini, ♦♦$p < 0.01$ as compared with OA- or LA-treated acini.

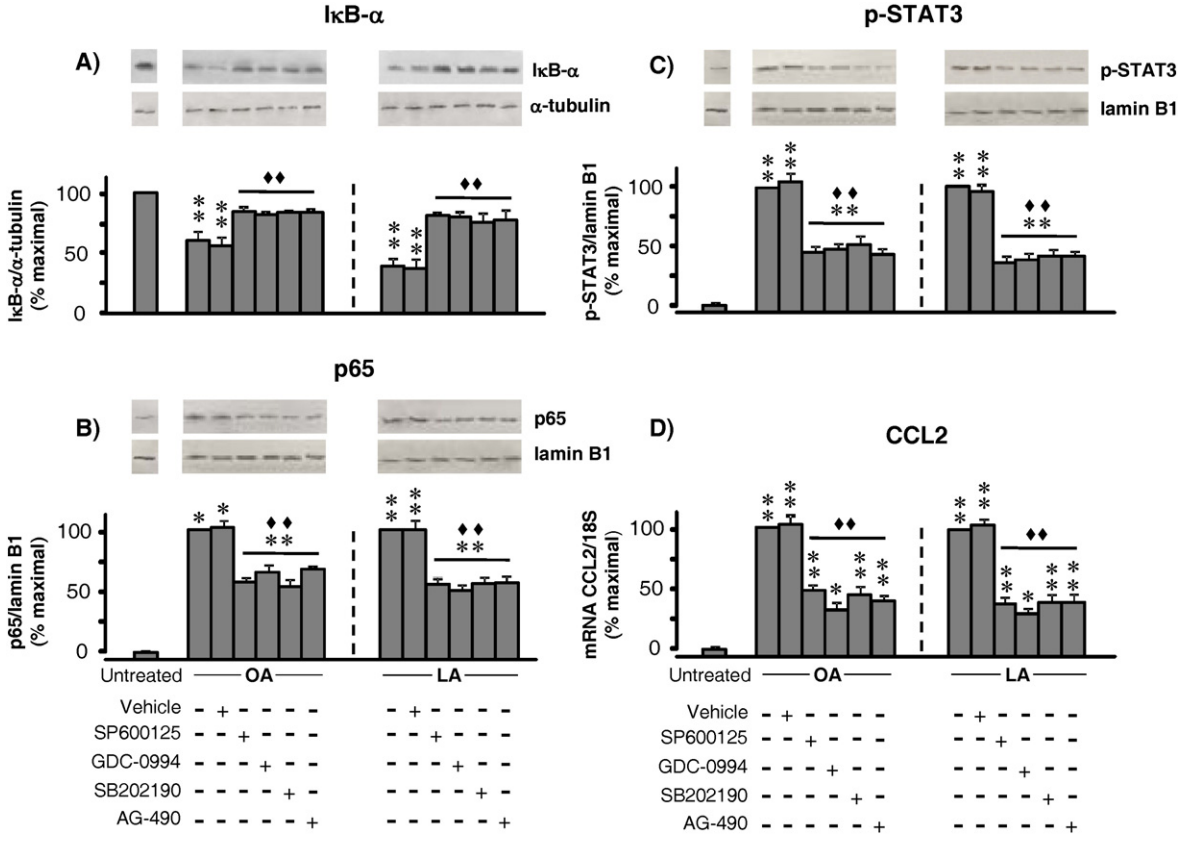


Fig. 5. MAPKs and JAK are upstreams of UFA-induced CCL2 up-regulation transcriptionally mediated by NF-κB and STAT3. Pancreatic acini exposed for 1 h to 300 μM of oleic acid (OA) or linoleic acid (LA) were pre-treated for 45 min with the vehicle (DMSO) or SP600125 (50 μM), GDC-0994 (50 μM) or SB202190 (50 μM), inhibitors of JNK-, ERK- and p38-MAPKs, respectively, or AG-490 (50 μM), JAK inhibitor. Levels of cytosolic IκB-α (A), nuclear p65 (B), and nuclear p-STAT3 (C) were analyzed by western-blot. Representative gels and densitometric analysis of the band intensity are shown. Densities (arbitrary units) were normalized with those of α-tubulin or lamin B1 (loading control for cytosolic or nuclear proteins, respectively). CCL2 mRNA expression (D) was analyzed by qRT-PCR with 18S as internal standard. Results (means ± SEM) from five experiments are expressed as percentages of the maximal response. $^{*}p < 0.05$, $^{**}p < 0.01$ as compared with untreated acini, ♦♦$p < 0.01$ as compared with OA- or LA-treated acini.

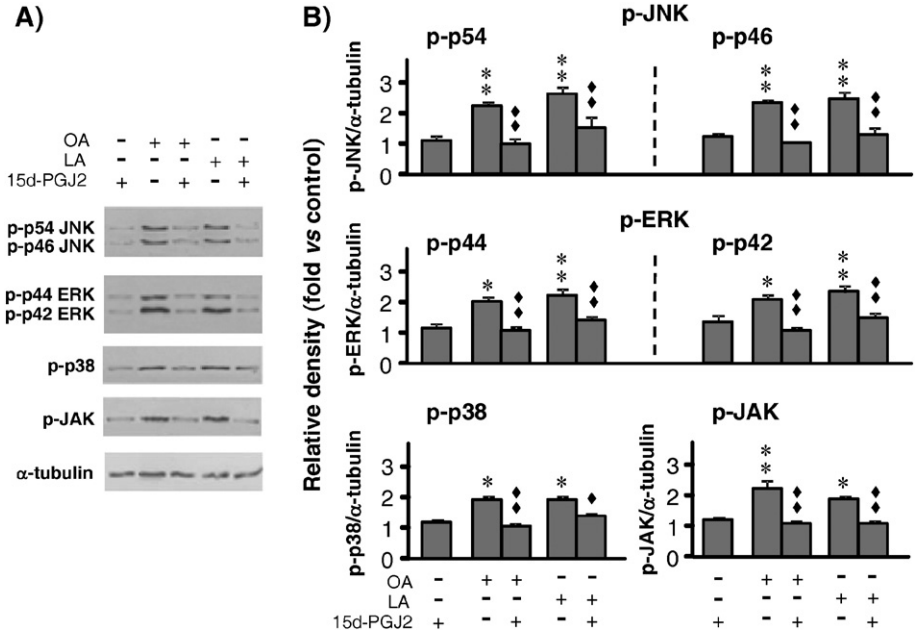


Fig. 6. 15-Deoxy-Δ(12,14)-prostaglandin J(2) (15d-PGJ2) inhibited UFA-mediated MAPKs and JAK activation. Pancreatic acini incubated for 1 h in the presence of 300 μM of the UFAs oleic acid (OA) or linoleic acid (LA) or EtOH, as the vehicle, (controls) were cultured with or without 15d-PGJ2 (10 μM). Phosphorylated MAPKs (p-MAPKs): c-Jun NH2-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), p38 and phosphorylated Janus kinase (p-JAK) were analyzed in cytoplasmic extracts by western blot. Representative gels of five experiments (A) and densitometric analysis of the band intensity (B) are shown. Densities (arbitrary units) were normalized with those of α-tubulin (loading control). Results (means ± SEM) are expressed as fold changes *vs* control acini. $^{*}p < 0.05$, $^{**}p < 0.01$ as compared with control acini, ♦$p < 0.05$, ♦♦$p < 0.01$ as compared with OA- or LA-treated acini.

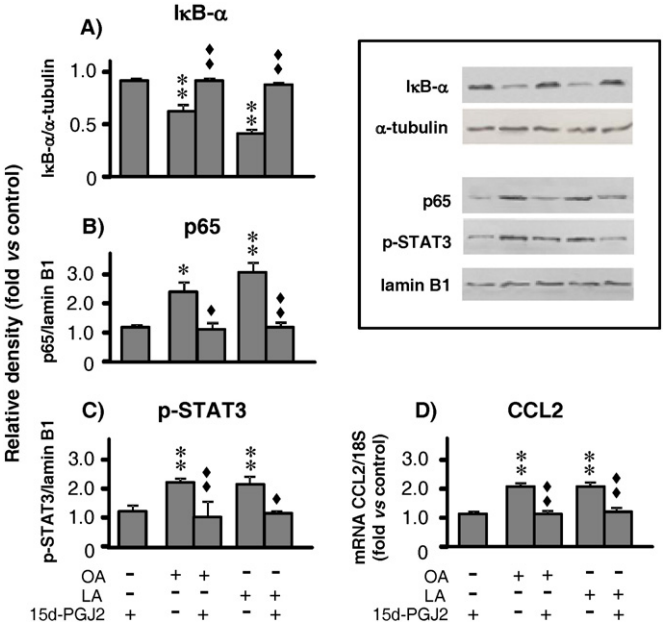


Fig. 7. 15-Deoxy-Δ(12,14)-prostaglandin J(2) (15d-PGJ2) suppressed UFA-induced CCL2 up-regulation *via* inhibition of NF-κB and STAT3 pathways. Pancreatic acini incubated for 1 h in the presence of 300 μM of the UFAs oleic acid (OA) or linoleic acid (LA) or EtOH, as the vehicle, (controls), were cultured with or without 15d-PGJ2 (10 μM). Representative western blots of five experiments and densitometric analysis of the band intensity of cytosolic IκB-α (A), nuclear p65 (B) and nuclear p-STAT3 (C) are shown. Densities (arbitrary units) were normalized with those of α-tubulin or lamin B1 (loading control for cytosolic or nuclear proteins, respectively). CCL2 mRNA expression (D) was analyzed by qRT-PCR with 18S as internal standard. Results (means ± SEM) are expressed as fold changes *vs* control acini. $^{*}p < 0.05$, $^{**}p < 0.01$ as compared with control acini, ♦$p < 0.05$, ♦♦$p < 0.01$ as compared with OA- or LA-treated acini.

phosphorylation [21,22]. However, the use of specific inhibitors for each kinase in the current study indicated that MAPKs as well as JAK are involved in the UFA-mediated activation of both NF-κB and STAT3 transcription factors.

The individual inhibition of these kinases did not totally repress the CCL2 overexpression induced by either OA or LA, suggesting that JNK-, p38- and ERK-MAPK and JAK must act in a synergic way on the mechanisms that converge in the CCL2 expression. In addition, the selective inhibition of NF-κB and STAT3 activation provides evidence that both transcription factors contribute to up-regulate mRNA CCL2 expression in acinar cells stimulated by OA or LA. In line with our results, the involvement of NF-κB in the FA-induced expression of pro-inflammatory genes has also been reported in other endothelial cells [15,23,24] and adipocytes [25]. However, NF-κB non-dependent mechanisms have also been demonstrated to mediate inflammatory response in dendritic cells [26] and macrophages [27].

Regarding the STAT3 signal, the current study reinforces the previously reported notion of the involvement of this pathway in the acinar inflammatory response to lipid components of necrotic adipose tissue of rats with severe AP [18]. Also, STAT3 activation has demonstrated to be involved in the inflammatory response of acinar cells during AP [28,29]. Taken together, this indicates that in addition to NF-κB and AP-1 [20,30,31], the transcriptional activity of STAT3 might be key in the signaling mechanisms that lead to the expression of inflammatory mediators in acinar cells exposed to stressful conditions.

Peroxisome proliferator-activated receptors (PPARs) are a family of ligand-inducible transcription factors involved in the lipid-associated inflammatory response [11]. PPARγ has been found to be highly expressed in pancreatic AR42J cells [32] and pancreatic tissue [33]. By acting as PPARγ ligands, n-3 PUFAs exerted suppressive effects on inflammatory response [34]. Conversely, the pro-inflammatory effects induced on macrophages by FAs proved to be the result of interference in the PPARγ anti-inflammatory activity [27]. This interference might be discarded in pancreatic acinar cells since the activation of the PPARγ pathway by adding 15d-PGJ2 in the presence of OA or LA resulted in the total inhibition of the UFA-mediated CCL2 up-regulation. In accordance with this result, PPARγ might not be considered to play a role in the acinar inflammatory response mediated by UFAs. However, the use of 15d-PGJ2, the most specific PPARγ agonist [35], allowed us to elucidate the signaling mechanisms triggered by the PPARγ pathway to inhibit the UFA-mediated CCL2 overexpression in pancreatic acini. Our data showed that PPARγ activation prevented the phosphorylation of MAPKs and JAK and hence the subsequent NF-κB and STAT3 activation. Consistent with our results, blockage of NF-κB [36] and STAT3 [37] activation has also been reported in cerulein-stimulated pancreatic AR42J cells pre-treated with 15d-PGJ2. The current data reinforce the notion that the use of PPARγ agonists might be considered as a potential therapeutic strategy for reducing inflammation during AP [38–40].

In line with our results, CCL2 overexpression in response to LA has been reported in different cell types [15,41,42], including acinar cells [13]. However, pro-inflammatory [16], anti-inflammatory [15] and no effects [17] have been described for OA. The pro-inflammatory action of OA found in the current study could be the basis to explain the etiopathology of AP experimentally induced in rats by retrograde perfusion of oleic acid into the pancreatic duct [43,44], such that the CCL2 up-regulation induced by OA in acinar cells might be the signal to attract circulating leukocytes to the gland and trigger severe forms of AP.

Hawkins et al. [45] reported that the lipid peroxidation and death of pancreatic acinar cells in response to FAs are related to the number of double bonds in the molecule. This finding led us in a recent study [18] to suggest that increases in PUFA/MUFA ratio found in necrotic adipose tissue of rats with severe AP could be responsible for the acinar inflammatory response induced by the total free FAs. Data shown in the current study leads us to dismiss this hypothesis since the CCL2 expression in acinar cells exposed to effective doses of either OA or LA proved to be similar.

Regarding the SFA effects, our study showed that both SA and PA failed to trigger inflammatory response in acinar cells. In line with previous studies [13,46,47], our findings corroborate the notion that the unsaturated state of FAs determines the injury and the inflammatory response in pancreatic acinar cells. Taken the UFA and SFA data together, our study provides evidence that the presence of bonds in the FA molecule, but not the number of double bonds nor the length of the chain, is the feature that determines the triggering of mechanisms required for the development of the inflammatory response in pancreatic acinar cells.

Our results suggest that UFAs would play a key role in AP such that they could be the link between obesity and a worse AP outcome. This notion is supported by a highly pertinent study by Noel et al. [5] who reported that UFAs, found in high concentrations in necrotic collections from obese patients with AP, are responsible not only for acinar necrosis, but also for triggering multisystem organ failure by mechanisms non-dependent on pancreatic necrosis. All these findings reinforce the notion that treatments preventing the generation of UFAs could have beneficial effects in severe AP.

5. Conclusion

This study contributes new information about the cellular mechanisms by which FAs exert differential effects on the inflammatory response triggered in pancreatic acinar cells depending on the unsaturation state. Our data provide mechanistic evidence that the most abundant UFAs, but not the major SFAs, in peripancreatic fat and released during AP, could play a role in the pathophysiology of AP. They are capable of up-regulating the acinar expression of CCL2 through the activation of MAPK/JAK-mediated NF-κB and STAT3 pathways. By targeting both transcription factors, the use of PPARγ agonists could be indicated as potential therapy in AP.

Competing interests

The authors declare no competing financial interests.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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