Biochimica et Biophysica Acta 1792 (2009) 1205–1211

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Mechanisms of dexamethasone-mediated chemokine down-regulation in mild and severe acute pancreatitis☆

S. Yubero, L. Ramudo, M.A. Manso, I. De Dios *

Department of Physiology and Pharmacology, University of Salamanca, 37007 Salamanca, Spain

ARTICLE INFO

Article history:
Received 2 September 2009
Received in revised form 28 September 2009
Accepted 1 October 2009
Available online 7 October 2009

Keywords:
Acute pancreatitis
Chemokines
Dexamethasone
MAPK
NF-κB
STAT3

ABSTRACT

This study aimed to investigate the role of therapeutic dexamethasone (Dex) treatment on the mechanisms underlying chemokine expression during mild and severe acute pancreatitis (AP) experimentally induced in rats. Regardless of the AP severity, Dex (1 mg/kg), administered 1 h after AP, reduced the acinar cell activation of extracellular signal-regulated kinase (ERK) and c-Jun-NH_2-terminal kinase (JNK) but failed to reduce p38-mitogen-activated protein kinase (MAPK) in severe AP. In both AP models, Dex inhibited the activation of nuclear factor-kappaB (NF-κB) and signal transducers and activators of transcription (STAT) factors. All of this resulted in pancreatic down-regulation of the chemokines monocyte chemoattractant protein-1 (MCP-1) and cytokine-induced neutrophil chemoattractant (CINC). Lower plasma chemokine levels as well as decreased amylasemia, hematocrit and plasma interleukin-1β (Il-1β) levels were found either in mild or severe AP treated with Dex. Pancreatic neutrophil infiltration was attenuated by Dex in mild but not in severe AP. In conclusion, by targeting MAPKs, NF-κB and STAT3 pathways, Dex treatment down-regulated the chemokine expression in different cell sources during mild and severe AP, resulting in decreased severity of the disease.

1. Introduction

Chemokines are important inflammatory mediators during (AP) involved in the activation and migration of leukocytes into the tissues [1]. On the structural basis, chemokines have been divided into different groups according to the position of the first two cysteine residues [2]. CC chemokines (e.g. MCP-1) act as chemoattractants and activators of non-polymorphonuclear leukocytes and CXC (e.g. CINC) as chemoattractants of neutrophils. Increased plasma chemokine levels have been reported in clinical [3,4] and experimental settings [2,5-7] of AP. Overexpression of chemokines has been found in pancreas of animals with experimentally induced AP [5,6,8,9] and also isolated acinar cells were shown to produce chemokines as an early signal in AP [6,10,11]. In addition, infiltrating leukocytes within the pancreas [9] and circulating leukocytes [12] have also been reported as cellular sources of chemokine production during AP. The type of chemokine and pattern of expression vary in the different models of AP and are not always related to the severity of the disease [6,8,10].

Glucocorticoids are used as anti-inflammatory and immunosuppressive agents in the treatment of inflammatory diseases by interfering with the synthesis and activation of pro-inflammatory factors. Nuclear factor-kappaB (NF-κB) and activator protein-1 (AP-1) have shown to be targets for glucocorticoid-mediated repression of inflammatory genes [13–15]. Also, interaction between glucocorticoids and signal transducers and activators of transcription (STAT) factors regarding cytokine production has recently been reported [16,17]. In addition, inhibition [18,19] and activation [20] of MAPK signalling by glucocorticoids have also been reported in *in vitro* studies; however, whether MAPKs play a significant role in the anti-inflammatory action of glucocorticoids has not yet been defined in vivo. Controversial results have been obtained when corticosteroids have been applied in AP [12,21-30] and they are not currently standard treatment for the disease. A better understanding of the mechanism of action in AP would help to consider their use as therapeutic strategy. On this basis, our aim was to examine the effect of dexamethasone (Dex), a potent anti-inflammatory synthetic corticosteroid, on the underlying signal pathways involved in the acinar expression of two structurally different chemokines, MCP-1 and CINC, during AP and to assess the consequences in the progression of the disease. The effectiveness of the Dx treatment has been evaluated both in mild and severe models of AP.

2. Materials and methods

2.1. Chemicals

Dexamethasone 21-phosphate disodium salt (Dex), taurocholic acid sodium salt hydrate, aminoacid mixture, bovine serum albumin (BSA), collagenase type XI, soybean trypsin inhibitor (STI), *N*-(2-

☆ Potential conflicts of interest do not exist.

* Corresponding author. Departamento Fisiología y Farmacología. Edificio Departamental, Campus Miguel de Unamuno, 37007 Salamanca, Spain. Fax: +34 923 294673.
E-mail address: bel@usal.es (I. De Dios).

0925-4439/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbadis.2009.10.001

hydroxyethyl) piperazine-N′-(2-ethanesulfonic acid) (HEPES), streptomycin and penicillin solution, Nonidet P-40, aprotinin, leupeptin, pepstatin, antipain, chymostatin, phenylmethanesulfonyl fluoride (PMSF), dithiothreitol (DTT) and buprenorphine, were supplied by Sigma Chemical Co. (Madrid, Spain). MCP-1 and Il-1β enzyme-linked immunosorbent assay (ELISA) kits were supplied by Bender MedSystems (Vienna, Austria) and CINC ELISA kit was by R&D Systems (Minneapolis, MN, USA). Agarose was supplied by Iberlabo (Madrid, Spain). All other standard analytical grade laboratory reagents were obtained from Merck (Madrid, Spain).

2.1.1. Animal models of pancreatitis and treatment

Male Wistar rats (250–300 g) were housed individually in cages and maintained at 22 ± 1 °C using a 12-h light/dark cycle. The animals were fasted overnight before the experiment but they were allowed free access to water. All experiments were performed in accordance with European Community guidelines on ethical animal research, established by the European Community (86/609/EEC). The study was approved by the Institutional Animal Care and Use Committee of the University of Salamanca (Spain).

Under anaesthesia with 2%–3% isofluorane, Forane® (Abott, Madrid, Spain), mild AP was induced by bile-pancreatic duct obstruction (BPDO), as previously described [31] and severe AP by retrograde infusion of 3.5% sodium taurocholate (NaTc) into the bile-pancreatic duct according to the method of Aho and Nevalainen [32]. Dexamethasone (Dex) was administered at a dose of 1 mg/kg by intramuscular injection 1 h after inducing AP. Studies were carried out at the following time periods: 3 h and 12 h in rats with BPDO-induced AP and 3 h and 6 h in rats with NaTc-induced AP. Sixteen rats were allocated in each experimental group and they were in turn distributed in three subgroups to collect different kinds of samples: cell lysates ($n = 5$), RNA ($n = 5$), pancreatic tissue and blood ($n = 6$). Postoperative analgesia was maintained by intramuscular injections of buprenorphine (0.2 mg/kg). At the end of the experiments the pancreas and blood samples were collected.

2.1.2. Analysis of phospho-MAPKs and IκBα

2.1.2.1. Preparation of total cell lysates. Acinar cells were isolated by collagenase digestion at 20 °C as previously described [33]. They were homogenized on ice in HEPES buffer, 10 mM, pH 7.9, containing 2 mM EDTA, and 25 mM KCl and supplemented with 1 mM PMSF and a protease inhibitor cocktail containing aprotinin, leupeptin, pepstatin, antipain and chymostatin (5 μg/ml each). The mixture was maintained on ice for 20 min, after which Nonidet P-40 (0.4%) was added for 2 min and then centrifuged at 4 °C for 3 min at 14,000 × *g*. The supernatants were collected and immediately stored at –80 °C until use.

2.1.2.2. Western blot analysis. Cytoplasmic extracts from acinar cells (40 μg) were separated by 12% SDS–PAGE and electrophoretically transferred to a nitrocellulose membrane. 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) non-fat dry milk for 1 h. Afterwards, blots were incubated overnight at 4 °C with the primary antibody (at a 1:1000 dilutions in TBS buffer pH 7.6, containing 0.1% (v/v) Tween 20 and 5% (w/v) BSA) against either each of the three phospho-MAPKs: p38, extracellular signal-regulated kinase (ERK1/2) and c-Jun NH_2-terminal kinase (JNK), IκBα or β-actin (Cell Signalling Technology, Beverly, MA). β-actin was used as the load control of protein. After washing for 1 h 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) non-fat dry milk and finally they were developed for visualization. The bands were detected with the Phototope-HRP Detection kit (Cell Signalling 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.

2.2. Determination of NF-κB- and STAT3-DNA binding

2.2.1. Nuclear cell extract preparation

Nuclear protein extracts were obtained from acinar cells using a commercial nuclear extract kit following the recommendations of the manufacturer (Active Motif, Rixensart, Belgium) and as was previously described [12].

NF-κB- and STAT3-DNA binding was measured in nuclear extracts with the respective ELISA-based commercial kits (NF-κB p65 and STAT3 TransAM assays, Active Motif, Rixensart, Belgium). Nuclear proteins (5 μg), were added to each well coated with an oligonucleotide containing the consensus binding site for either NF-κB or STAT3 and incubated for 1 h. Activation was detected by incubation for 1 h with the respective primary antibody: anti-NF-κB, which specifically recognizes an epitope (p65) accessible only when the factor is activated and bound to its target DNA and anti-STAT3, which recognizes epitopes only accessible when STAT3 is activated. A secondary anti-IgG horseradish peroxidase conjugate allows detection of the activated NF-κB and STAT3 by a colorimetric reaction. Absorbance was read within 5 min at 450 nm with a reference wavelength of 655 nm.

2.3. Analysis of mRNA expression for MCP-1 and CINC

Total RNA was extracted from isolated acinar cells and pancreas using RNAeasy kit treated with amplification grade DNase 1 (Quiagen, Valencia, Spain) according to the manufacturer's instructions. Purity of RNA was verified by ethidium bromide staining on 1% agarose gels. The purity of RNA was assessed by a 260/280 ratio and the integrity of RNA was verified by the presence of well-defined 28S and 18S rRNA bands.

Semiquantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) was performed to analyze mRNA expression of MCP-1 and CINC in acinar cells and pancreas. Total RNA (1 μg) was reversed transcribed by using 1st Strand cDNA synthesis kit (Roche). The cDNA synthesized was used as template for PCR amplification by using Taq DNA polymerase, dNTPack (Roche). The following primer pairs (Roche) were used: MCP-1 (sense: 5′-CACTATGCAGGTCTCTGTCACG-3′, antisense; 5′-GACTCACTTGGTTCTGGTCCA-3′, product size: 294 bp), CINC (sense: 5′-CTCCAGCCACACTCCAACAGA-3, antisense: 5′-CACCCTAACACAAAACACGAT-3′, product size: 600 bp). Oligonucleotide primers for β-actin (sense: 5′-CACGGCATTGTAACCAACTG-3′, antisense: 5′-TCTCAGCTGTGGTGGTGAAG-3′, product size: 400 bp) were used as internal control. PCR was performed following procedures: 30 cycles of denaturation at 94 °C for 30 s, annealing 56 °C for 45 s and elongation at 72 °C for 45 s. The amplified PCR products were separated on a 2% agarose gel stained with ethidium bromide. The MCP-1, CINC and β-actin PCR products were run together on the same gel in order to normalize the band densities to the β-actin band which were densitometrically quantified with a Gel Doc 1000/2000 image analysis system (BioRad) using the QuantityOne software programme.

2.3.1. Blood analysis

Hematocrit was measured in blood samples. Amylase activity was analyzed in plasma according to the method of Hickson [34], as well as IL-1β, MCP-1 and CINC concentrations using the respective ELISA kits strictly following manufacturers' recommendations. All samples were run in duplicated and averaged.

2.4. Myeloperoxidase (MPO) determination

Neutrophil infiltration was estimated in pancreas by measuring tissue MPO activity, following the method of Bhatia and Hegde [7]. MPO activity is expressed as fold increase over controls.

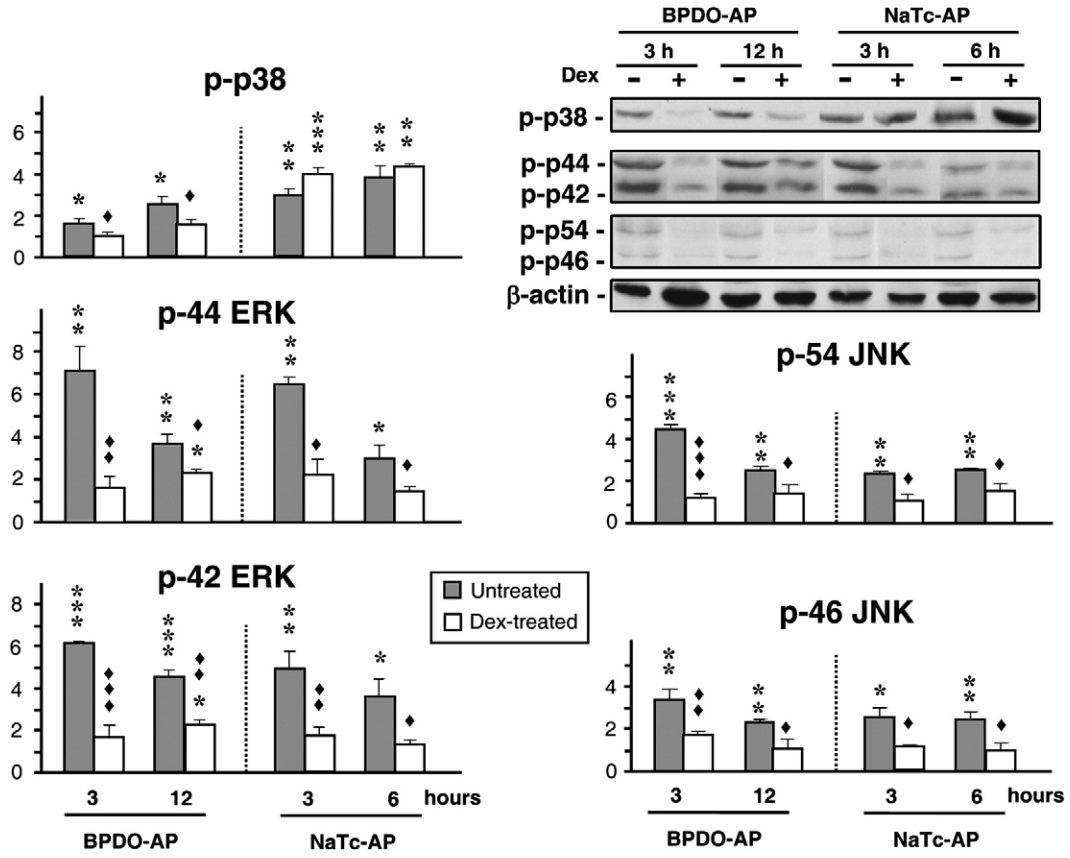

Fig. 1. Phosphorylation of p38, extracellular signal-regulated kinases (ERK) and Jun N-terminal kinases in acinar cells of controls and rats with acute pancreatitis (AP) induced either by bile-pancreatic duct obstruction (BPDO) or by 3.5% sodium taurocholate (NaTc), untreated or treated with dexamethasone (Dex) given 1 h after AP. Representative Western blots of five experiments are shown. β-actin was used as loading control. Relative intensity of the bands in each group vs controls was considered in densitometric analysis. Results shown are the means ± SEM. ANOVA followed by the Dunnett test showed significant differences vs controls (*$p<0.05$, **$p<0.01$, ***$p<0.01$) and vs untreated AP ($^{\blacklozenge}p<0.05$, $^{\blacklozenge\blacklozenge}p<0.01$, $^{\blacklozenge\blacklozenge\blacklozenge}p<0.001$).

2.5. Statistical analysis

Results are expressed as mean ± SEM. Statistical analysis was carried out using the analysis of variance (ANOVA) followed by the Dunnett test to evaluate at each time point differences between controls, untreated AP and Dx-treated AP. *P* values lower than 0.05 were considered to be significant.

3. Results

3.1. Effect of dexamethasone on acute pancreatitis-induced MAPK activity

The significant increase in ERK and JNK phosphorylation found in acinar cells from early stages of AP was significantly reduced by Dex treatment either in BPDO- or NaTc-induced AP. However, only in mild but not in severe AP did Dex administration inhibited p38 phosphorylation. (Fig. 1).

3.2. Effect of dexamethasone on acute pancreatitis-induced NF-κB and STAT3 activity

Analysis of NF-κB activation, evaluated by IκBα cytosolic concentrations (Fig. 2A) and p65 nuclear levels (Fig. 2B), revealed that Dex administration maintained IκBα at control levels and inhibited NF-κB-DNA binding either in BPDO- or NaTc-induced AP.

Both in the mild and the severe model of AP, STAT3 activity was significantly ($p<0.05$) attenuated in pancreatic acinar cells by Dex treatment (Fig. 3).

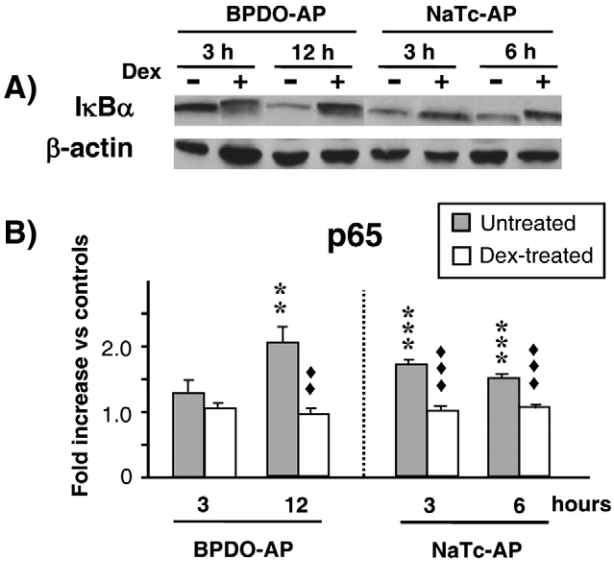

Fig. 2. NF-κB activation in acinar cells of controls and rats with acute pancreatitis (AP) induced either by bile-pancreatic duct obstruction (BPDO) or by 3.5% sodium taurocholate (NaTc), untreated or treated with dexamethasone (Dex) given 1 h after AP. (A) Representative Western blot of five experiments of IκBα and β-actin, as loading control. (B) Mean values ± SEM of p65 nuclear levels (measured as fold increase vs controls) of five experiments. ANOVA followed by the Dunnett test showed significant differences vs controls (**$p<0.01$, ***$p<0.001$) and vs untreated AP ($^{\blacklozenge\blacklozenge}p<0.01$, $^{\blacklozenge\blacklozenge\blacklozenge}p<0.001$).

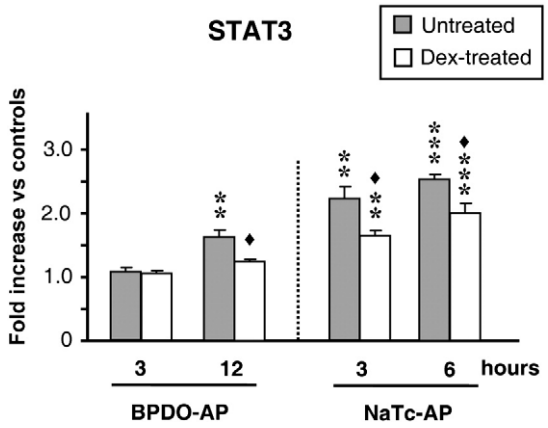

Fig. 3. STAT3 activity in acinar cells of rats with acute pancreatitis (AP) induced either by bile-pancreatic duct obstruction (BPDO) or by 3.5% sodium taurocholate (NaTc), untreated or treated with dexamethasone (Dex) given 1 h after AP. Number of animals in each group and AP period: 5. Results (fold increase vs controls) are shown as the means ± SEM. ANOVA followed by the Dunnett test showed significant differences vs controls (**$p<0.01$, ***$p<0.001$) and vs untreated AP (♦$p<0.05$).

3.3. Effect of dexamethasone on chemokine expression

The overexpression of MCP-1 and CINC mRNA found in pancreatic acinar cells of rats with AP from early stages was significantly inhibited by Dex treatment either in BPDO or NaTc AP model (Fig. 4).

Analysis of chemokine expression in pancreatic tissue (Fig. 5) revealed a significant repression of the AP-induced MCP-1 and CINC mRNA up-regulation in response to Dex administration, both in mild and severe pancreatitis.

As Fig. 6 shows, the high levels of MCP-1 found in plasma of rats with AP were significantly reduced by Dex treatment both in mild and severe AP. Dex treatment was effective to significantly ($p<0.05$) reduce CINC plasma levels during BPDO-induced AP and at early stages in the NaTc model.

3.4. Effect of dexamethasone on the severity of acute pancreatitis

Hyperamylasemia and IL-1β plasma levels were significantly reduced by Dex administration, both in mild and severe AP (Fig. 7).

As Table 1 shows, hematocrit values were significantly reduced by Dex administration during BPDO-induced AP and at early stages of NaTc-induced AP. Neutrophil infiltration in pancreas, evaluated by MPO activity, was significantly ($p<0.01$) reduced by Dex treatment in BPDO-induced AP, but no effect was found in the severe AP model.

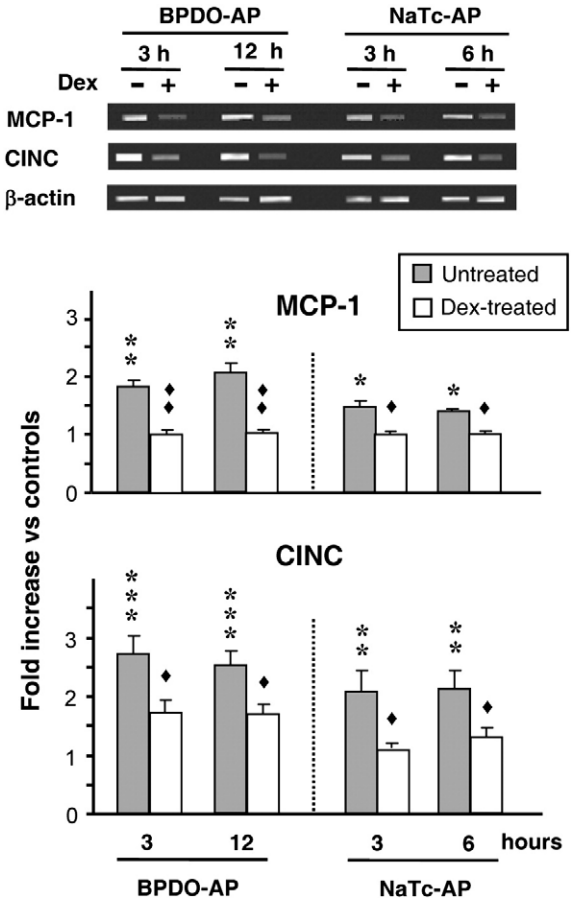

Fig. 4. mRNA expression of monocyte chemoattractant protein-1 (MCP-1) and cytokine-induced neutrophil chemoattractant (CINC) in acinar cells of rats with acute pancreatitis (AP) induced either by bile-pancreatic duct obstruction (BPDO) or by 3.5% sodium taurocholate (NaTc), untreated or treated with dexamethasone (Dex) given 1 h after AP. A representative RT-PCR and the mean values ± SEM (measured as fold increase vs controls of MCP-1/β-actin and CINC//β-actin ratios). Number of animals in each group and AP period: 5. ANOVA followed by the Dunnett test showed significant differences vs controls (*$p<0.05$, **$p<0.01$, ***$p<0.001$) and vs untreated AP (♦$p<0.05$, ♦♦ $p<0.01$).

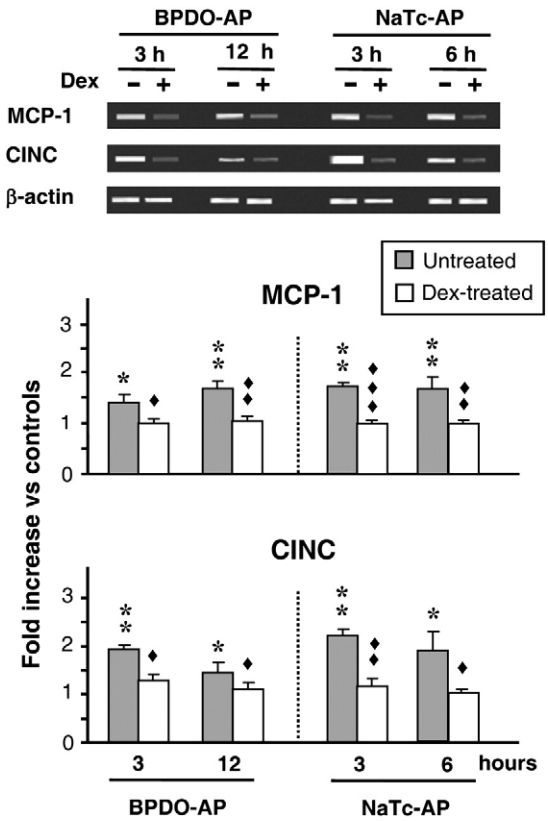

Fig. 5. mRNA expression of monocyte chemoattractant protein-1 (MCP-1) and cytokine-induced neutrophil chemoattractant (CINC) in pancreatic tissue of rats with acute pancreatitis (AP) induced either by bile-pancreatic duct obstruction (BPDO) or by 3.5% sodium taurocholate (NaTc), untreated or treated with dexamethasone (Dex) given 1 h after AP. A representative RT-PCR and the mean values ± SEM (measured as fold increase vs controls of MCP MCP-1/β-actin and CINC//β-actin ratios). Number of animals in each group and AP period: 5. ANOVA followed by Dunnett test showed significant differences vs controls (*$p<0.05$, **$p<0.01$) and vs untreated AP (♦$p<0.05$, ♦♦$p<0.01$, ♦♦♦$p<0.001$).

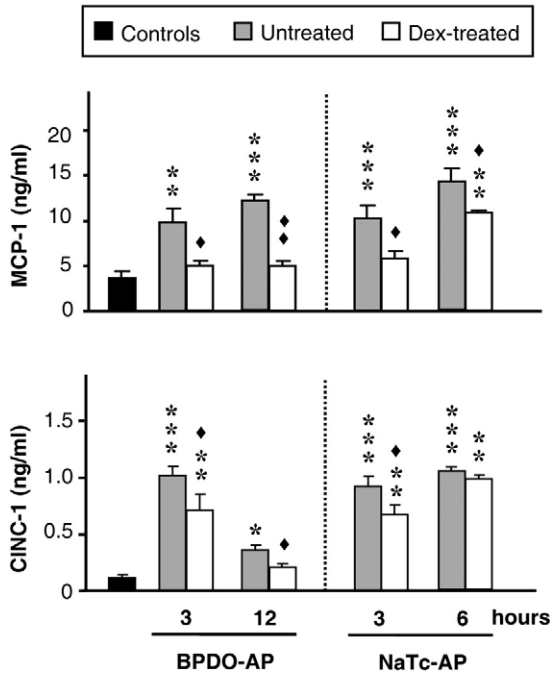

Fig. 6. Plasma monocyte chemoattractant protein-1 (MCP-1) and cytokine-induced neutrophil chemoattractant (CINC) concentrations in controls and rats with acute pancreatitis (AP) induced either by bile-pancreatic duct obstruction (BPDO) or by 3.5% sodium taurocholate (NaTc), untreated or treated with dexamethasone (Dex) given 1 h after AP. Number of animals in each group and AP period: 6. Results shown are the means ± SEM. ANOVA followed by the Dunnett test showed significant differences vs controls (* $p<0.05$, ** $p<0.01$, *** $p<0.01$) and vs untreated AP (♦ $p<0.05$, ♦♦ $p<0.01$).

4. Discussion

It is widely accepted that AP triggered by pancreatic activation of trypsinogen within acinar cells [35] results in pancreatic damage followed by a local and systemic inflammatory response in which different mediators play a key role. On this basis the use of glucocorticoids has been proposed because of their ability to interfere with the cytokine network [36]. However, little attention has been focused on studying the effects of glucocorticoids on the mechanisms involved in the expression of chemokines, despite the fact they have been shown to influence the leukocyte migration into the tissues and to act as important regulators of leukocyte activation in situ [1]. The effectiveness of glucocorticoids for the treatment of AP has been a matter of dispute. Prophylactic administration has generally shown to have protective effects on the injury induced in AP [21-26]; however, both beneficial [27,28] and inefficient [29,30] results have been reported for therapeutic treatments.

Our results indicated that Dex, given 1 h after inducing AP, at a dose equivalent to that used in humans with AP [25], was able to reduce the pancreatic expression of MCP-1 and CINC in rats with either mild or necrotizing AP. Evidence that pancreatic acinar cells themselves are able to activate MAPKs, NF-κB and STAT3 as signalling mechanisms mediating chemokine expression has been previously reported [5,6,11,16,37]. According to our results, these down-stream pathways are targeted by Dex treatment, leading to a reduction in the pancreatic acinar chemokine expression during AP. Regarding MAPK activity, we demonstrate that irrespective of the severity of AP Dex reduces JNK and ERK phosphorylation. However, only in mild but not in severe AP was Dex treatment was able to inhibit p38 activity. Failure in the Dex action on p38MAPK activity has been previously reported in *in vitro* experiments using either lipopolysaccharide-stimulated macrophages [19] or pancreatic acinar cells stimulated with pancreatitis-associated ascitic fluid [16]. It could be speculated that different upstream MAPK-kinases (MKKs) may be not equally affected by Dex action. However, our study allows to compare two settings in which p38MAPK activity is differently influenced by Dex treatment in accordance with the severity of AP. Environmental stress, inflammatory cytokine and growth factors strongly activate the three MAPKs. However, they have shown different behaviour in response to ischemia–reperfusion. For an unknown reason JNK is not activated during ischemia, but rather during reperfusion, whereas p38 is activated during ischemia and remains active during reperfusion [38,39]. Given that the BPDO model induces mild AP and NaTc model necrotizing and hemorrhagic AP, we suggest that Dex treatment was unable to hinder p38 MAPK activity in the severe AP model due to the serious circulation impairment developed within the pancreas.

MAPKs have been demonstrated to act as upstream signals of NF-κB and STAT3 activity [16,37,40], by promoting the phosphorylation of the inhibitory protein IκBα and STAT3, respectively. Thus, the negative cross-talk between Dex and MAPKs, may contribute, at least in part, to reducing the activation of both transcription factors found in Dex-treated rats. In addition, glucocorticoids are considered anti-inflammatory agents by directly acting at transcriptional level to repress genes encoding inflammatory factors. Two different mechanisms have been reported for glucocorticoids to explain their ability to repress NF-κB activation: cross-coupling with p65 containing Rel-related protein complexes and induction of IκBα synthesis [13,14]. Our results showed that Dex treatment up-regulated the synthesis of IκBα inhibitory protein, thus maintaining NF-κB complex sequestered in the cytoplasm complex. The fact that control values of p65 at nuclear level were found in Dex-treated rats with either mild or necrotizing AP is evidence of failure in the NF-κB translocation to the nucleus. Inhibition of NF-κB activation by methylprednisolone [26] or Dex [28] treatments in other AP models has been previously reported. As a result of the negative cross-talk between Dex and the transcription factors, down-regulation of chemokine expression was found both in rats with mild or severe AP treated with Dex. These results are in line with those obtained by Ramudo et al. [16] who, using isolated acinar cells stimulated by pancreatitis-associated ascitic

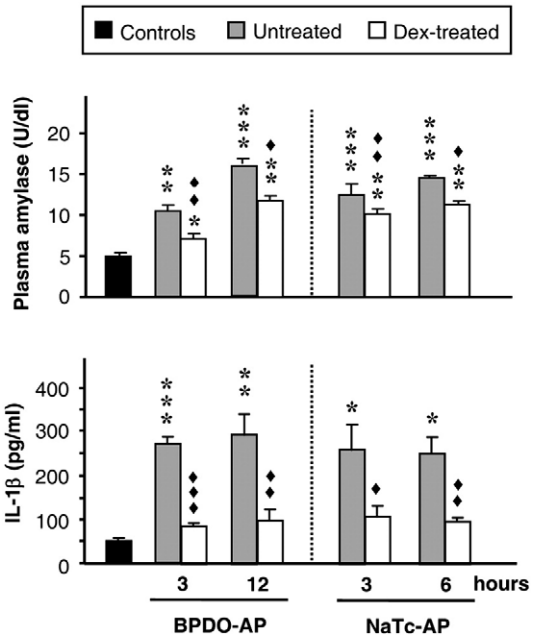

Fig. 7. Plasma amylase activity and Interleukin 1-β (IL-1β) in controls and rats with acute pancreatitis (AP) induced either by bile-pancreatic duct obstruction (BPDO) or by 3.5% sodium taurocholate (NaTc), untreated or treated with dexamethasone (Dex) given 1 h after AP. Number of animals in each group and AP period: 6. Results shown are the means ± SEM. ANOVA followed by the Dunnett test showed significant differences vs controls (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) and vs untreated AP (♦ $p<0.05$, ♦♦ $p<0.01$, ♦♦♦ $p<0.001$).

Table 1
Hematocrit and myeloperoxidase (MPO) activity in pancreatic tissue in controls and rats with acute pancreatitis (AP) induced either by bile-pancreatic duct obstruction (BPDO) or by 3.5% sodium taurocholate (NaTc), untreated or treated with dexamethasone (Dex).

	Controls	BPDO-AP				NaTc AP			
		3 h		12 h		3 h		6 h	
		–	Dex	–	Dex	–	Dex	–	Dex
Hematocrit (%)	43.72 ± 0.38	49.59 ± 0.65 a ($p<0.01$)	46.50 ± 0.29 a ($p<0.01$) b ($p<0.01$)	48.86 ± 0.54 a ($p<0.01$)	44.77 ± 0.72 b ($p<0.01$)	54.49 ± 0.26 a ($p<0.001$)	51.47 ± 0.70 a ($p<0.01$) b ($p<0.01$)	54.52 ± 0.88 a ($p<0.01$)	53.83 ± 1.58 a ($p<0.01$)
MPO (fold increase vs controls)	1.00	0.94 ± 0.27	1.12 ± 0.11	5.20 ± 0.46 a ($p<0.01$)	1.52 ± 0.31 b ($p<0.01$)	8.50 ± 1.67 a ($p<0.01$)	8.60 ± 1.01 a ($p<0.01$)	12.96 ± 1.49 a ($p<0.001$)	11.45 ± 0.95 a ($p<0.01$)

Number of animals in each group: 6. Results shown are the means ± SEM. ANOVA followed by the Dunnett test showed significant differences vs controls (a) and untreated AP (b).

fluid, have recently reported an effective inhibition of NF-κB- and STAT3-mediated MCP-1 up-regulation by Dex.

In addition to acinar cells, chemokines may also be produced by other cell types residing within the pancreas. Endothelial vascular cells [41], stellate cells [42] and infiltrated leukocytes [9] may over-express MCP-1 and CINC in response to local and circulating cytokines, which would act as activating factors of NF-κB [43] and STAT3 [44] transcription factors. Given that IL-1β was found to be reduced in rats with AP treated with Dex, we suggest that chemokine down-stream pathways might be impaired in these cell types due to the cytokine down-regulation exerted by Dex treatment. Thereby, Dex, given after inducing AP, becomes able to repress the pancreatic chemokine expression during mild and severe AP, not only in acinar but also in non-acinar cells.

Nevertheless, the analysis of MPO activity revealed that Dex treatment was able to reduce the neutrophil infiltration in rats with mild AP but failed in the severe model of AP. We suggest that the intense oxidative stress triggered in the pancreas of rats with NaTc-induced AP [6] may reduce the effectiveness of Dex in repressing the leukocyte recruitment, given the powerful chemoattractant effect of the reactive oxygen species [45].

The action of Dex treatment attenuating MAPK, NF-κB and STAT3 activity and thereby down-regulating chemokine expression results in protection against AP as is also evidenced by lower hyperamylasemia and hematocrit values, findings which indicate certain preservation of pancreatic exocrine function and less accumulation of circulating fluid in the tissues (edema), respectively. These effects could be explained by some protective effects attributed to glucocorticoids besides the anti-inflammatory action, such as circulation improvement [25] and stabilization of cytoskeleton proteins [46].

In summary, data obtained in two experimental models of AP shows that Dex, with an early therapeutical window, may be considered a potential strategy in the treatment of the disease by targeting key signalling pathways in chemokine expression and thereby preventing the progression of AP.

Acknowledgements

This study was supported by a grant from FEDER-FIS (Fondo de Investigación Sanitaria), Spain (PI08/0035). Thanks are due to Elizabeth Nestor for her linguistic assistance.

References

[1] D.H. Adams, A.R. Lloyd, Chemokines: leukocyte recruitment and activation cytokines, Lancet 349 (1997) 490–495.
[2] PM. Murphy, The molecular biology of leukocyte chemoattractant receptors, Annu. Rev. Immunol. 12 (1994) 593–633.
[3] S. Regnér, S. Appelros, C. Hjalmarsson, J. Manjer, J. Sadic, A. Borgstrom, Monocyte chemoattractant protein 1, active carboxypeptidase B and CAPAP at hospital admission are predictive markers for severe acute pancreatitis, Pancreatology 8 (2008) 42–49.
[4] J.W. Naskalski, B. Kusnierz-Cabala, J. Panek, B. Kedra, Poly-C specific ribonuclease activity correlates with increased concentrations of IL-6, IL-8 and sTNFR55/sTNFR75 in plasma of patients with acute pancreatitis, J. Physiol. Pharmacol. 54 (2003) 439–448.
[5] I. Samuel, M.A. Yorek, A. Zaheer, R.A. Fisher, Bile-pancreatic juice exclusion promotes Akt/NF-kappaB activation and chemokine production in ligation-induced acute pancreatitis, J Gastrointest. Surg. 10 (2006) 950–959.
[6] S. Yubero, L. Ramudo, M.A. Manso, I. De Dios, The role of redox status on chemokine expression in acute pancreatitis, Biochim. Biophys. Acta 1792 (2009) 148–154.
[7] M. Bhatia, A. Hegde, Treatment with antileukinate, a CXCR2 chemokine receptor antagonist, protects mice against acute pancreatitis and associated lung injury, Regul. Pept. 138 (2007) 40–48.
[8] M. Brady, M. Bhatia, S. Christmas, M.T. Boyd, J.P. Neoptolemos, J. Slavin, Expression of the chemokines MCP-1/JE and cytokine-induced neutrophil chemoattractant in early acute pancreatitis, Pancreas 25 (2002) 260–269.
[9] J. Sun, M. Bhatia, Blockade of neurokinin-1 receptor attenuates CC and CXC chemokine production in experimental acute pancreatitis and associated lung injury, Am. J. Physiol. Gastrointest. Liver Physiol. 292 (2007) G143–153.
[10] M. Bhatia, M. Brady, Y.K. Kang, E. Costello, D.J. Newton, S.E. Christmas, J.P. Neoptolemos, J. Slavin, MCP-1 but not CINC synthesis is increased in rat pancreatic acini in response to cerulein hyperstimulation, Am. J. Physiol. Gastrointest. Liver Physiol. 282 (2002) G77–85.
[11] R.D. Ramnath, J. Sun, S. Adhikari, M. Bhatia, Effect of mitogen-activated protein kinases on chemokine synthesis induced by substance P in mouse pancreatic acinar cells, J. Cell. Mol. Med. 11 (2007) 1326–1341.
[12] S. Yubero, L. Ramudo, M.A. Manso, I. De Dios, Targeting peripheral immune response reduces the severity of necrotizing acute pancreatitis, Crit. Care Med. 37 (2009) 240–245.
[13] N. Auphan, J.A. DiDonato, C. Rosette, A. Helmberg, M. Karin, Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis, Science 270 (1995) 286–290.
[14] R.I. Scheinman, A. Gualberto, C.M. Jewell, J.A. Cidlowski, A.S Baldwin Jr, Characterization of mechanisms involved in transrepression of NF-kappa B by activated glucocorticoid receptors, Mol. Cell. Biol. 15 (1995) 943–953.
[15] H.F. Yang-Yen, J.C. Chambard, Y.L. Sun, T. Smeal, T.J. Schmidt, J. Drouin, M. Karin, Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction, Cell 62 (1990) 1205–1215.
[16] L. Ramudo, S. Yubero, M.A. Manso, S. Vicente, I. De Dios, Signal transduction of MCP-1 expression induced by pancreatitis-associated ascitic fluid in pancreatic acinar cells, J. Cell. Mol. Med. 7 (2009) 1314–1320.
[17] C. Unterberger, K.J. Staples, T. Smallie, L. Williams, B. Foxwell, A. Schaefer, B. Kempkes, T.P. Hofer, M. Koeppel, M. Lohrum, H. Stunnenberg, M. Frankenberger, L. Ziegler-Heitbrock, Role of STAT3 in glucocorticoid-induced expression of the human IL-10 gene, Mol. Immunol. 45 (2008) 3230–3237.
[18] L. Bladh, K. Johansson-Haque, I. Rafter, S. Nilsson, S. Okret, Inhibition of extracellular signal-regulated kinase (ERK) signaling participates in repression of nuclear factor (NF)-κB activity by glucocorticoids, Biochim. Biophys. Acta 1793 (2009) 439–446.
[19] J.L. Swantek, M.H. Cobb, T.D. Geppert, Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) is required for lipopolysaccharide stimulation of tumor necrosis factor alpha (TNF-α) translation: glucocorticoids inhibit TNF-alpha translation by blocking JNK/SAPK, Mol. Cell. Biol. 17 (1997) 6274–6282.
[20] A.Q. Qi, J. Qui, L. Xiao, Y.Z. Chen, Rapid activation of JNK and p38 by glucocorticoids in primary cultured hippocampal cells, J. Neurosci. Res. 80 (2005) 510–517.
[21] M.O. Osman, N.O. Jacobsen, J.U. Kristensen, C.G. Larsen, S.L. Jensen, Beneficial effects of hydrocortisone in a model of experimental acute pancreatitis, Dig. Surg. 16 (1999) 214–221.
[22] A. Paszt, T. Takács, Z. Rakonczay, J. Kaszaki, A. Wolfard, L. Tiszlavicz, G. Lázár, E. Duda, K. Szentpáli, L. Czakó, M. Boros, A. Balogh, G. Jr, Lázár. The role of the glucocorticoid-dependent mechanism in the progression of sodium taurocholate-induced acute pancreatitis in the rat, Pancreas 29 (2004) 75–82.
[23] B. Gloor, W. Uhl, O. Tcholakov, A. Roggo, C.A. Muller, M. Worni, M.W. Büchler, Hydrocortisone treatment of early SIRS in acute experimental pancreatitis, Dig. Dis. Sci. 46 (2001) 2154–2161.
[24] G. Lázár Jr., J. Varga, G. Lázár, E. Duda, T. Takács, A. Balogh, J. Lonovics, The effects of glucocorticoids and a glucocorticoid antagonist (RU 38486) on experimental acute pancreatitis in rat, Acta Chir. Hung. 36 (1997) 190–191.

[25] Z.F. Wang, C. Liu, Y. Lu, R. Dong, J. Xu, L. Yu, Y.M. Yao, Q.G. Liu, C.E. Pan, Dexamethasone and dextran 40 treatment of 32 patients with severe acute pancreatitis, World J. Gastroenterol. 10 (2004) 1333–1336.
[26] Z. Rakonczay Jr., E. Duda, J. Kaszaki, B. Iványi, I. Boros, J. Lonovics, T. Takács, The anti-inflammatory effect of methylprednisolone occurs down-stream of nuclear factor-kappaB DNA binding in acute pancreatitis, Eur. J. Pharmacol. 464 (2003) 217–227.
[27] X.P. Zhang, L. Zhang, Y. Wang, Q.H. Cheng, J.M. Wang, W. Cai, H.P. Shen, J. Cai, Study of the protective effects of dexamethasone on multiple organ injury in rats with severe acute pancreatitis, J. Pancreas 8 (2007) 400–412.
[28] X.P. Zhang, L. Zhang, H.M. Xu, Y.P. Xu, Q.H. Cheng, J.M. Wang, H.P. Shen, Application of tissue microarrays to study the influence of dexamethasone on NF-kappaB expression of pancreas in rat with severe acute pancreatitis, Dig. Dis. Sci. 53 (2008) 571–580.
[29] T. Kimura, G.D. Zuidema, J.L. Cameron, Acute pancreatitis. Experimental evaluation of steroid, albumin and trasylol therapy, Am. J. Surg. 140 (1980) 403–408.
[30] L.I. Cosen-Binker, M.G. Binker, G. Negri, O. Tiscornia, Experimental model of acute pancreatitis in Wistar rat. Glucocorticoid treatment profile, Dig. Dis. Sci. 48 (2003) 1453–1464.
[31] S. Sevillano, A.M. De la Mano, I. De Dios, L. Ramudo, M.A. Manso, Major pathological mechanisms of acute pancreatitis are prevented by *N*-acetylcysteine, Digestion 68 (2003) 34–40.
[32] H.J. Aho, T.J. Nevalainen, Experimental pancreatitis in the rat. Ultrastructure of sodium taurocholate-induced pancreatic lesions, Scand. J. Gastroenterol. 15 (1980) 417–424.
[33] L. Ramudo, M.A. Manso, S. Sevillano, I. de Dios, Kinetic study of TNF-alpha production and its regulatory mechanisms in acinar cells during acute pancreatitis induced by bile-pancreatic duct obstruction, J. Pathol. 206 (2005) 9–16.
[34] J.C. Hickson, The secretion of pancreatic juice in response to stimulation of the vagus nerves in the pig, J. Physiol. 206 (1970) 275–297.
[35] S.D. Leach, I.M. Modlin, G.A. Scheele, F.S. Gorelick, Intracellular activation of digestive zymogens in rat pancreatic acini. Stimulation by high doses of cholecystokinin, J. Clin. Invest. 87 (1991) 362–366.
[36] T. Wilckens, R. De Rijk, Glucocorticoids and immune function: unknown dimensions and new frontiers, Immunol. Today 18 (1997) 418–424.
[37] R.D. Ramnath, M. Bhatia, Substance P treatment stimulates chemokine synthesis in pancreatic acinar cells via the activation of NF-kappaB, Am. J. Physiol. Gastrointest. Liver Physiol. 291 (2006) G1113–1119.
[38] M.A. Bogoyevitch, J. Gillespie-Brown, A.J. Ketterman, S.J. Fuller, R. Ben-Levry, A. Ashworth, C.J. Marshall, P.H. Sugden, Stimulation of the stress-activated mitogen-activated protein kinase subfamilies in perfused heart. P38/RK mitogen-activated protein kinases and c-Jun N-terminal kinases are activated by ischemia/reperfusion, Circ. Res. 79 (1996) 162–173.
[39] J.M. Kyriakis, Signalling by the germinal center kinase family of protein kinases, J. Biol. Chem. 274 (1999) 5259–5262.
[40] J. Turkson, T. Bowman, J. Adnane, Y. Zhang, J.Y. Djeu, M. Sekharam, D.A. Frank, L.B. Holzman, J. Wu, S. Sebti, R. Jove, Requirement for Ras/Rac1-mediated p38 and c-Jun N-terminal kinase signaling in Stat3 transcriptional activity induced by the Src oncoprotein, Mol. Cell. Biol. 19 (1999) 7519–7528.
[41] E. Mordelet, H.A. Davies, P. Hillyer, I.A. Romero, D. Male. Chemokine transport across human vascular endothelial cells, Endothelium 14 (2007) 7–15.
[42] A. Masamune, N. Suzuki, K. Kikuta, M. Satoh, K. Satoh, T. Shimosegawa, Curcumin blocks activation of pancreatic stellate cells, J. Cell. Biochem. 97 (2006) 1080–1093.
[43] H.P. Hohmann, R. Remy, B. Pöschl, A.P. van Loon, Tumor necrosis factors-alpha and -beta bind to the same two types of tumor necrosis factor receptors and maximally activate the transcription factor NF-kappa B at low receptor occupancy and within minutes after receptor binding, J. Biol. Chem. 265 (1990) 15183–15188.
[44] K. Takeda, S. Akira, STAT family of transcription factors in cytokine-mediated biological responses, Cytokine Growth Factor Rev. 11 (2000) 199–207.
[45] B. Poch, F. Gansauge, B. Rau, U. Wittel, S. Gansauge, A.K. Nüssler, M. Schoenberg, H.G. Beger, The role of polymorphonuclear leukocytes and oxygen-derived free radicals in experimental acute pancreatitis: mediators of local destruction and activators of inflammation, FEBS Lett. 461 (1999) 268–272.
[46] F. Castellino, J. Heuser, S. Marchetti, B. Bruno, A. Luini, Glucocorticoid stabilization of actin filaments: a possible mechanism for inhibition of corticotropin release, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 3775–3779.