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Chemerin alleviates acute pancreatitis in the rat thorough modulation of NF-κB signal

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ABSTRACT

Objective: Chemerin, an adipokine, works as the chemoattractant for the immune cells. The role of chemerin in the inflammatory reaction is controversial. Chemerin has been shown to aggravate the inflammatory response, but other studies demonstrated its anti-inflammatory influence. This study assessed the effects of chemerin on acute pancreatitis (AP) *in vivo* and *in vitro*.
Methods: For *in vivo* experiments male Wistar rats were used. For *in vitro* study rat pancreatic AR42J cells were employed. Chemerin (1, 5 or 10 μg/kg) was given to the rats prior to the induction of AP by subcutaneous caerulein infusion (25 μg/kg). For *in vitro* studies cells were subjected to caerulein (10 nM) with or without chemerin (100 nM). Serum amylase activity was measured by enzymatic method, serum TNFα concentration - by ELISA kit. Western-blot was used to examine cellular proteins.
Results: AP was confirmed by histological examination. Chemerin given to AP rats decreased histological manifestations of AP, reduced serum amylase activity and TNFα concentration. In AR42J cells subjected to caerulein with addition of chemerin signal for TNFα was reduced comparing to the cultures treated with caerulein alone. Analysis of the dynamics of nuclear translocation for p50, p65 and Bcl-3 points out to NF-κB attenuation as a mechanism of observed anti-inflammatory action of chemerin.
Conclusion: Chemerin significantly alleviated severity of AP in the rat, this is possibly due to the inhibition of pro-inflammatory signaling in the pancreatic cells.

Introduction

Acute pancreatitis is an inflammatory nonbacterial disease of the pancreatic gland, pathogenesis of which remains still unclear. The main patho-mechanism responsible for the development of acute pancreatitis traditionally has been believed that is related to the intracellular activation of trypsin and self-digestion of the pancreas. However recent studies are focused on the role of nuclear factor kappa B (NF-κB) in the progression of inflammatory process in the gland [1,2]. The local process of acute pancreatitis employing lysosomal and proteolytic enzymes, stimulation of inflammatory cells and release of pro-inflammatory cytokines such as: tumor necrosis factor α (TNFα), interleukins (IL-1β, IL-6, IL-8), gut hormones (peptide YY, gastric inhibitory peptide), together with generation of reactive oxygen and nitrogen species could be often transformed into systemic inflammation [3–5].

Chemerin acts as a potent chemoattractant, that recruits cells from bone marrow and induces migration of the immune cells, such as macrophages and natural killers toward the site of inflammation [6,7]. The main source of chemerin appears to be the adipose tissue and this substance is recognized now as one of adipokines (adipocytokines) [8]. Recent studies have shown that chemerin could be produced also in the liver, pancreas, intestinal

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epithelium, fibroblasts, skin epidermis, platelets, and in the respiratory tract [7,9–11].

On the other hand, chemerin has been presented as pro-inflammatory adipokine, since it was reported to aggravate experimental colitis [12], and its blood level was positively correlated with inflammation, obesity and insulin resistance [13–15]. High concentration of chemerin was found in the inflammatory exudates and elevated level of chemerin was observed in patients with Crohn's disease and rheumatoid arthritis [7,11,16,17].

Years of research have proved that NF-κB is expressed ubiquitously within various cell types. NF-κB binding site is found in the promoters/enhancers of a large number of different genes [18]. The canonical mechanism of NF-κB complex regulation constitutes of inhibitory proteins IκB (inhibitor of NF-κB) and specific IκB kinase (IKK) responsible for phosphorylation of IκB resulting in the release of active and ready for nuclear translocation NF-κB. In response to diverse external stimuli, numerous genes controlled by NF-κB are implicated in the regulation of immune response, stress reactions and apoptosis.

NF-κB is involved in the control of its own regulators, such as IκBα, p105, or A20, whose genes are regulated in NF-κB dependent manner [19,20]. In this way, active promotion of inhibitory IκBα gene expression by NF-κB creates a negative feedback regulatory loop influencing the duration of the NF-κB response. This mechanism was very precisely documented in terms of TNFα stimulation, showing that deficiency of IκBα remarkably prolonged the NF-κB response upon TNFα stimulation [21].

The NF-κB family comprises of five proteins (p105/p50 (NF-κB1), p100/52 (NF-κB2) and p65 (RelA), RelB, c-Rel). The NFκB family members possess the ability to form variety of any homo- or heterodimeric complexes of distinct regulatory activity with a pronounced predominance of p65/p50 complex in all cell types. In the opposition to the p65, Rel B and c-Rel proteins being mostly responsible for genes activation, p50 and p52 homo- and heterodimers, when bound to DNA, were found to repress transcriptional activity of genes being controlled in NFκB-dependent manner. Moreover, p50 and p52 might be aggregated with IκBζ or Bcl-3 (B-cell lymphoma 3-encoded protein) and on this way acquire transcriptional activation abilities. Their precursors, p105 and p100 respectively, may abolish the nuclear translocation of NF-κB when bound with it, what formally makes them the inhibitors of NF-κB [22]. Bcl-3 functions as a co-activator of gene expression when associated with p50 and p52 homodimers. Bcl-3 also increases occupancy of IκB binding site with p50 homodimer leading to the suppression of NF-κB target gene transcription. This shown dual role of Bcl-3 as a co-activator or suppressor [23].

This study was undertaken to evaluate the effect of chemerin administration on the course of acute pancreatitis in the rat and on the rat acinar pancreatic cell line AR42J, used as a model of pancreatic tissue to study possible mechanism of chemerin influence on intracellular NF-κB signal transduction pathway.

Methods

Materials

Following items were purchased: caerulein from Sigma Co, (St Louis, MO, USA), chemerin (CHEM RPA945Hu01) was from Cloud Clone Corp. (Huston, TX, USA), amylase reagent was from Alpha Diagnostic Warszawa, Poland), Vetbutal was from Biowet (Puławy, Poland). TNFα was measured using an ELISA commercial kit (R&D systems, Minneapolis MN, USA). Protein A-Agarose, primary antibodies and secondary HRP conjugated antibodies (horseradish peroxidase-conjugated) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). SuperSignal West Pico Chemiluminescent Substrate chemerin was from Sigma Co, (St Louis, MO, USA).

Study *in vivo*

Animals and experimental protocol

All procedures were performed in accordance with the policies regarding the use of laboratory animals and accepted by the Jagiellonian University Ethic Committee. Male Wistar rats (weight 220–250 g) were employed for the experiments. Animals were housed in cages under standard conditions, in a temperature-controlled environment with a 12/12 h light/dark cycle, with free access to food (commercial pellet chow) and water. Prior to the experiments, rats were deprived of food for 24 h, water was not limited. All experiments were carried out at the same time, in the morning. For the experiments, rats were placed in individual Bollman cages. Acute pancreatitis (AP) was induced by subcutaneous (s.c.) infusion of caerulein at total dose of 25 μg/kg for 5 hs. Caerulein was diluted in saline and infused at a rate of 1 mL/h (5 μg/kg/h). Control groups received 0.5 mL of vehicle saline injected intraperitoneally (i.p.), followed 30 min later by s.c. infusion of 0.9% saline for 5 h. Chemerin (1, 5, or 10 μg/kg) was dissolved in 0.9% saline and given in a volume of 0.5 mL to the rats as a bolus i.p. injection 30 min prior to the start of caerulein administration.

Animals were randomly allocated to 8 separate groups:

1. Control group – rats that received 0.5 mL of vehicle i.p. followed by s.c. infusion of 0.9% saline for 5 h (n = 6 rats),
2. Chemerin 1 control group – rats given 1 μg/kg of chemerin before saline infusion (n = 4 rats), Chemerin 5 control group – rats given 5 μg/kg of chemerin before saline infusion (n = 4 rats),
3. Chemerin 10 control group – rats given 10 μg/kg of chemerin before saline infusion, (n = 4 rats),
4. Acute pancreatitis group (AP) – rats that received s.c. infusion of caerulein (n = 8 rats),
5. Chemerin 1 + AP group – rats given 1 μg/kg of chemerin before s.c. infusion of caerulein (n = 8 rats),
6. Chemerin 5 + AP group – rats given 5 μg/kg of chemerin before s.c. infusion of caerulein (n = 8 rats),
7. Chemerin 10 + AP group – rats given 10 μg/kg of chemerin before s.c. infusion of caerulein (n = 8 rats),

Biochemical parameters

After 5 h of experiment rats were subjected to pentobarbital anesthesia (0,06 g/kg i.p.). Blood samples were collected from the *vena cava* to measure serum amylase activity and TNFα level. Amylase activity was determined using the modified method with a special sacharogen reagent Alpha Diagnostic for quantitative determination of this enzyme. TNFα was determined using an ELISA kit, as previously reported [24]. The blood samples, were left for 2 h at room temperature for coagulation and then centrifuged (at 3500 rpm for 10 min). Serum samples were frozen and kept at −80 °C until assayed.

Pancreatic weight and histological examination

The pancreata were carefully resected, rinsed, put on the blotting paper to dry out and then weighted. Samples of pancreatic tissue were collected and subjected to histopathological assessment. For histological studies pancreatic samples were fixed in 10% buffered neutral formalin solution, processed, stained with hematoxylin/eosin and subjected to histopathological examination by a

professional histologist without knowledge of the treatment given. The histological grading of edema, neutrophil infiltration and vacuolization changes were assessed using scale from 0 to as described previously (for edema: 0 = no edema, 1 = interlobular edema, 2 = interlobular edema and moderate interlobular edema, 3 = interlobular edema and severe interlobular edema; for neutrophil infiltration, or vacuolization of acinar cells, from 0 = no infiltration, or vacuolization, to 3 = maximal alternations) [24].

In vitro study

Cell cultures

Pancreatic AR42J cells have been purchased from ATCC (American Type Culture Collection, Manassas, USA). Cells were subcultured weekly in MEM supplemented with 4.5 g/L glucose, 10% fetal bovine serum, 1% non-essential amino acids, penicillin and streptomycin (50 U/mL) (Sigma, Aldrich). For the experiment $1x10^6$ AR42J cells were seeded on a 10 mm diameter dish in MEM with addition of 0.5% fetal bovine albumin with supplementation of antibiotics. For stimulation chemerin at concentrations of 1, 10 or 100 nM was added, without or with addition of caerulein (10 nM). For further *in vitro* studies only 100 nM of chemerin was selected as an experimental point with observed the most spectacular and relevant response of the cells. AR42J cell incubated in the presence of caerulein (10 nM) alone were used as a relative AP control. Control cells were incubated without any investigated substances.

Protein fractionation

After 48 h of incubation with stimulating factors cells were harvested using rubber scraper in ice cold PBS (phosphate buffered saline) and collected by short centrifugation at 4 °C. Then cell pellets were resuspended in 400 μL of extraction buffer A containing 10 mM Hepes, 10 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA (ethylenediamine tetraacetic acid), 1 mM DTT (dithiothreitol), 0.1 mM PMSF (phenylmethylsulphone fluoride) pH 7.4 and kept on ice for 15 min. Subsequently, samples were added with 25 μL 10% NP-40, mixed vigorously and centrifuged at 14 000×*g* for 15 s at 4 °C. The supernatant containing cytosolic fraction of proteins was removed and stored at −80 °C until further analysis. Pellets containing cellular nuclei were resuspended in 50 μl extraction buffer C containing 50 mM Hepes, 50 mM KCl, 300 mM NaCl, 1 mM EDTA, 10% glycerol, 1 mM DTT, 0.1 mM PMSF, pH 7.8 kept on ice for 20min and shake every 5 min. Subsequently, samples were centrifuged at 14 000×*g* for 10 min at 4 °C and the supernatants containing nuclear fraction of proteins were transferred to the fresh Eppendorf tubes and stored at −80 °C until further analysis.

The protein concentration was confirmed by employing the BCA Protein Assay (Thermo Fisher Scientific, USA) according to the manufacturer's protocol.

Immunoblotting

Samples of cytoplasmic or nuclear proteins have been suspended in 10 μl of Western blot sample buffer (50 mM Tris-HCl pH 6,8; 1 mM DTT, 2% SDS, 0,01% BB, 10% glycerol), boiled for 5 min at 95 °C, immediately cooled on ice and loaded on the 10% or 12% SDS-polyacrylamide gel and subjected to the electrophoretic separation. After separation samples were transferred onto the PVDF membrane (BioRad, USA). Following transfer each membrane was blocked for 2 h at room temperature with the blocking buffer (5% non-fat dried milk in PBS) and membranes were exposed for 1 h to the primary antibody diluted 1:1000 in TBST (0,1 M Tris pH 8.0; 150 mM NaCl; 0,5% TritonX-100). After exposure to antibodies solutions each membrane was washed three times for 15 min in TBST buffer. Following washes suitable secondary antibodies were applied in the dilution 1:5000 in TBST for 1 h at room temperature. All primary antibodies: GAPDH (glyceraldehyde 3-phosphate dehydrogenase,V-18) [sc-20357], NF-κB p50 (NLS) [sc-114], NF-κB p65 (C-20) [sc-372], Bcl-3 (C-14) [sc-185], TNF alpha (R-19), as well as secondary antibodies: goat anti-rabbit IgG-HRP [sc-2004], rabbit anty-goat IgG-HRP [sc-2768] were purchased from Santa Cruz Biotechnology (Santa Cruz, USA). Following the exposure to secondary antibodies the final washing procedure was performed as described previously protein bands were detected using Super-Signal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific Waltham, MA) according to the manufacturer's protocol. To document equal protein loading each blot was stripped and probed with GAPDH antibody. All presented results were obtained in at least three consecutive experiments [25]. Semi-quantitative optical density analysis was performed using G-Box EF (Synoptics, GB).

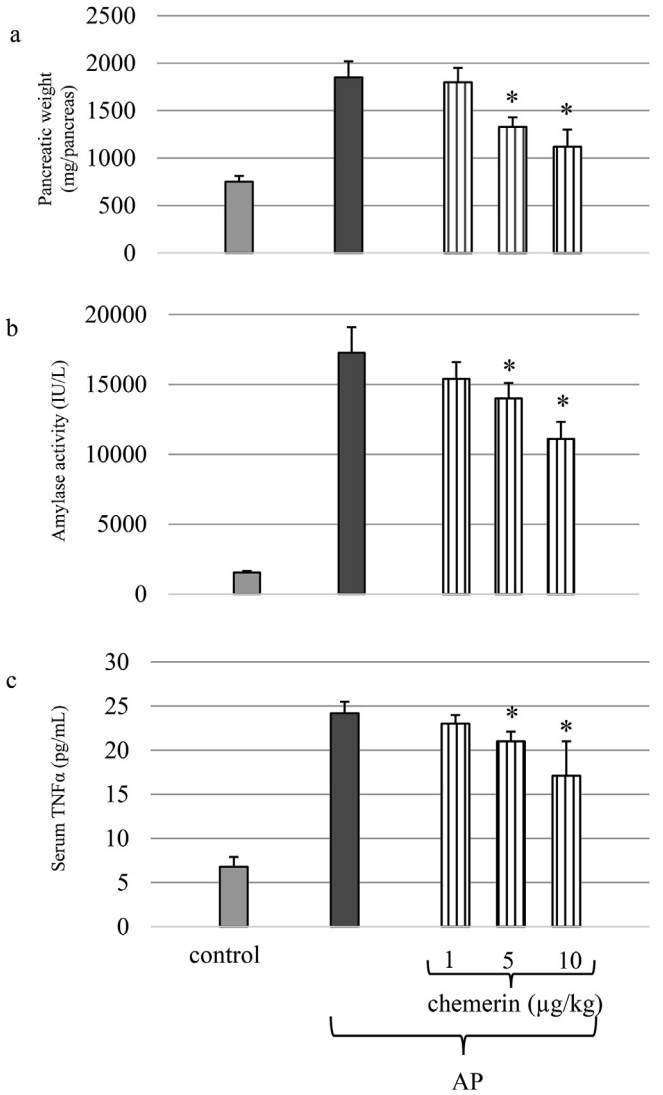

Fig. 1. Effects of chemerin (1, 5, or 10 μg/kg) on pancreatic weight (a), serum amylase activity (b), serum TNFα concentration (c) tissue in the rats subjected to caerulein-induced pancreatitis (AP). Control = intact animals. Means ± SEM from the separate experiments, each performed on 8 rats. Asterisk indicates statistically significant ($p < 0.05$) decrease below the values detected in the rats with AP alone.

Statistical analysis

Results are expressed as means ± SEM. Comparison of the differences between the mean values of various groups of experiments was made by analysis of variance or the Student's *t*-test for unpaired data and Wilcoxon test for paired data. For post hoc analysis the Neuman-Keuls test was engaged. Differences with a p value of <0.05 were considered statistically significant.

Results

In vivo study

The effects of chemerin on pancreatic weight, pancreatic morphology, serum amylase activity and serum TNFα concentration

In the control animals (Control group) mean pancreatic weight was 750 ± 60 mg, serum amylase level achieved 1550 ± 120 IU/L and TNFα concentration was 6.8 ± 1.1 pg/mL (Fig. 1). Chemerin alone given at doses of 1, 5, 10 μg/kg to the control rats failed to affect significantly pancreatic weight, pancreatic morphology, serum amylase activity or TNFα concentration and these data was no presented for the sake of clarity.

Subcutaneous infusion of caerulein to the rats produced acute pancreatitis (AP) in these animals. Pancreatic weight and amylase activity were significantly increased to 1850 ± 170 mg and 17 260 ± 1840 IU/L, respectively, whereas TNFα concentration rose to 24.2 ± 1.3 pg/mL (Fig. 1). In the pancreas of AP rats, characteristic signs of acute pancreatitis were observed: peritoneal fluid was accumulated, the pancreas was grossly swollen, inter- and intra-pancreatic edema were accompanied by perivascular infiltration of leukocytes and typical vacuolization of the acinar cells (Figs. 2 and 3). Application of chemerin to the rats prior to the caerulein infusion (Chemerin + AP group) resulted in the reduction of pancreatic edema, serum amylase activity, serum TNFα concentration and morphological signs of inflammation as compared to the animals with acute pancreatitis alone (Figs. 2 and 3). Chemerin given at dose of 10 μg/kg to the rats with acute pancreatitis significantly decreased pancreatic weight, serum amylase activity and serum TNFα concentration (to 1120 ± 180 mg/kg, 11 100 ± 1220 IU/L and 17.1 ± 3.9 pg/mL respectively) (Fig. 1).

***In vitro* study**

Based on the prior *in vivo* and *in vitro* observations we have selected only one dose (100 nM) of chemerin, which seemed to be the most efficient in the attenuation of caerulein induced inflammatory process, to be used in all *in vitro* experiments.

The effect of caerulein and chemerin on the expression of TNFα in AR42J cells

Immunoblotting analysis of cellular proteins isolated from AR42J cell revealed the substantial increase of TNFα synthesis in the cell cultures subjected to caerulein, while chemerin alone did not change TNFα protein abundance comparing to the control (Fig. 4, line 1, 2 and 3). But in the cells incubated with both caerulein and chemerin the expression level of TNFα protein drastically decreased (Fig. 4, line 4) when compared to the control and especially to the cells exposed to the caerulein alone (Fig. 4, line 2).

Dynamics of nuclear trafficking of p50 and p65 proteins in AR42J cells in response to caerulein and chemerin

To preserve the regulatory complexes of NF-κB, cell cultures were subjected to the mild lysis conditions employing buffers of low stringency with no reducing agents. Cytoplasmic as well as

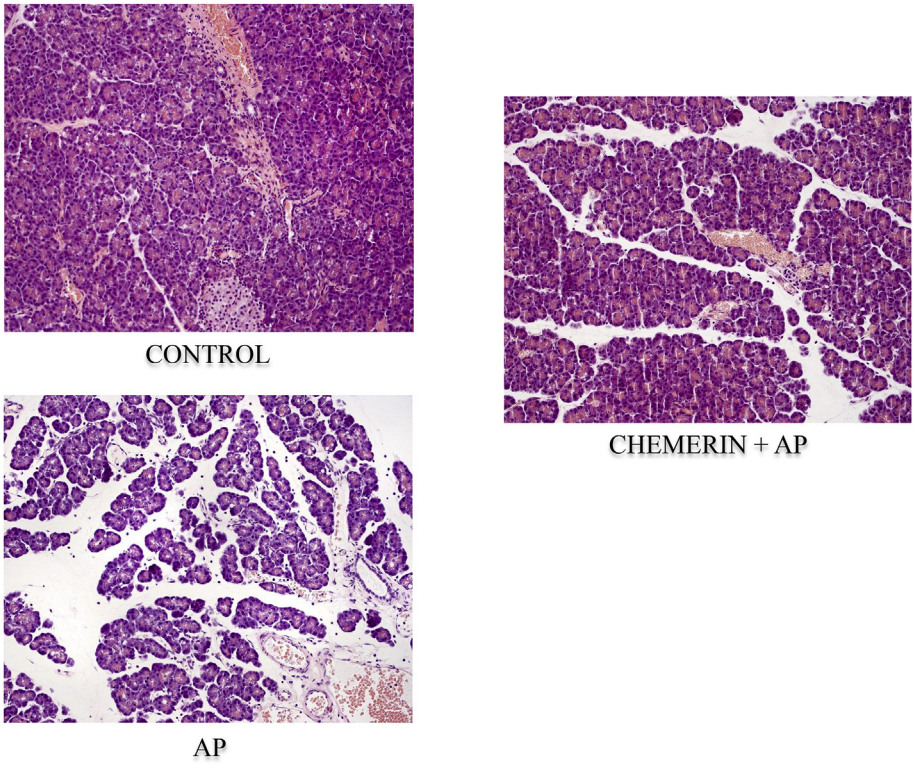

Fig. 2. Histological pictures of pancreas of rats exposed to caerulein-induced pancreatitis (AP) and AP rats pretreated with chemerin at dose of 10 μg/kg (AP + chemerin). Control = intact animals. Magnification 200x.

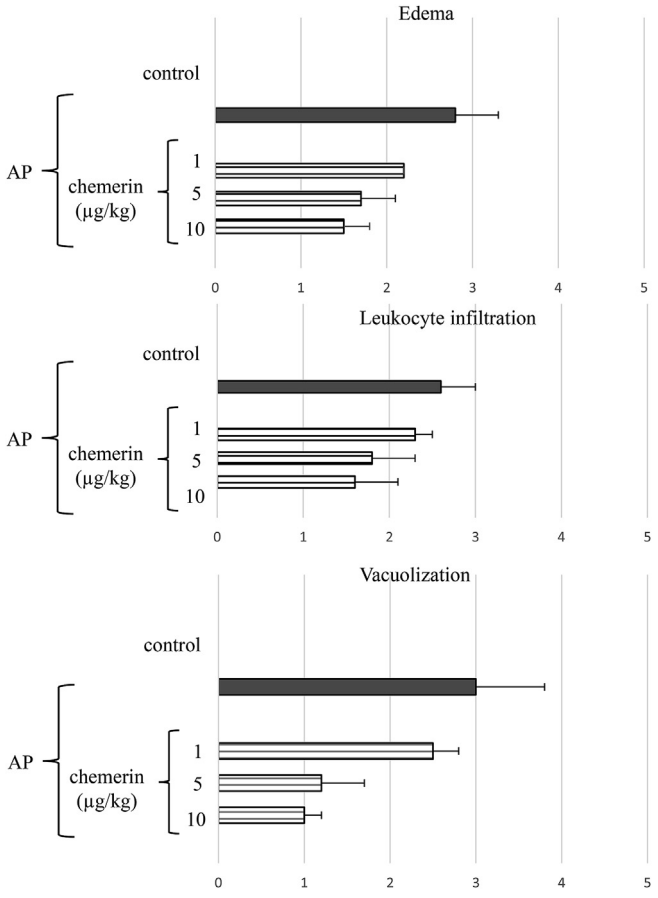

Fig. 3. Graphic presentation of morphological changes of the pancreas taken from control rats, from rats with acute pancreatitis (AP) and from AP rats pretreated with chemerin (1, 5, or 10 μg/kg). Asterisk indicates statistically significant s ($p < 0.05$) decrease below the values detected in the rats with AP alone.

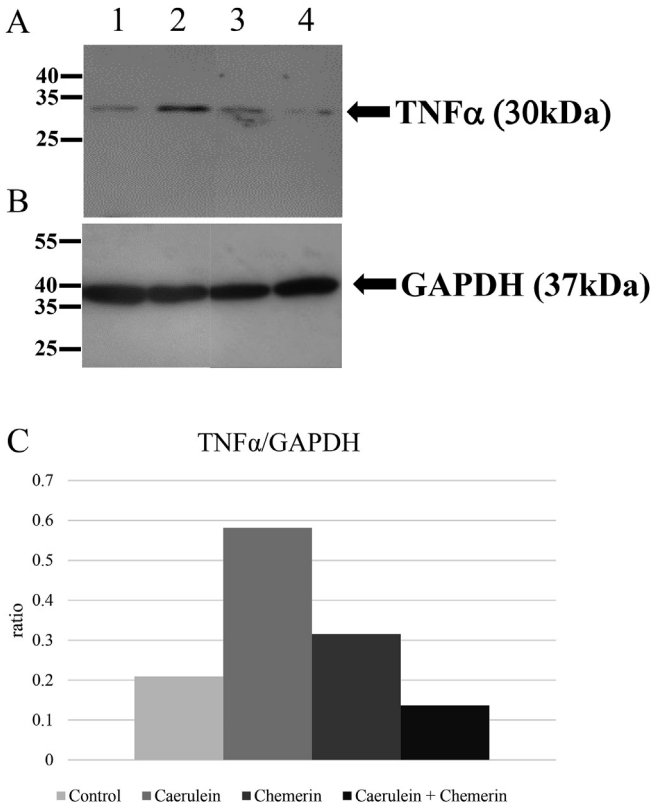

Fig. 4. Immunoblotting analysis of TNFα expression in proteins isolated from AR42J rat cell cultures. Protein samples (5 μg per line) were probed with antibody for TNFα (panel A). Line 1 – control cells, line 2 – cells incubated with caerulein (10 nM), line 3 – cells subjected to chemerin (100 nM) and line 4 – cells exposed to both factors caerulein (10 nM) and chemerin (100 nM). Blots shown in the figure were composed of two parts of the same exposure to remove unpresented data. Presented results were obtained in at least 3 consecutive experiments. The most representative results were picked for presentation. Blot has been re-probed with anti GAPDH (panel B) antibody to assess the equal load of samples on the lines. Values (panel C) were expressed as the ratio of the optical density of the tested protein band to the optical density value of the corresponding GAPDH band.

nuclear fraction have been isolated and analysed separately to study the dynamics of nuclear translocation of NF-κB components.

High cytoplasmic abundance of p50 was corresponding with low nuclear translocation in control cells (Fig. 5 A, B line 1, Fig. 5 H). Samples isolated from cells stimulated with caerulein showed no cytoplasmic and almost the same as described for the control nuclear abundance of p50 protein (Fig. 5 A, B line 2, Fig. 5 H). Stimulation of AR42J cultures with chemerin led to high level of p50 found in cytoplasm, as seen in the control, and high nuclear level of analysed protein (Fig. 5 A, B line 3, Fig. 5 H). Surprisingly, in the samples isolated from cells stimulated with both caerulein and chemerin we did not observe any sign of p50 in the cytoplasmic fraction and barely visible band corresponding with p50 protein in the nuclear fraction (Fig. 5 A, B line 4, Fig. 5 H).

Analysis of another canonical member of NF-κB complex – p65, reveals high and almost homogenous high cytoplasmic level of this protein in all samples collected from control as well as stimulated with caerulein, chemerin or their combination (Fig. 5 C lines 1–4, Fig. 5 H). Nuclear fraction showed almost equal and low amount of p65 protein in the samples isolated from control cell cultures, as well as, the cells stimulated with caerulein or chemerin alone (Fig. 5 D lines 1–3, Fig. 5 H) especially when compared to the cytoplasmic signal. Only cells subjected to combination of both factors manifested substantially lower abundance of analysed protein comparing to other samples (Fig. 5 D line 4, Fig. 5 H).

Results of immunoblotting analysis showed almost equal amount of Bcl-3 in cytoplasmic fraction of all analysed samples (Fig. 5 E lines 1–4, Fig. 5 H) with slight decrease observed in the cells stimulated with caerulein (Fig. 5 E line 2, Fig. 5 H). Different effect was observed in the nuclear fraction. Level of Bcl-3 in samples collected from caerulein stimulated cells was higher than that observed in control (Fig. 5 F lines 1, 2, Fig. 5 H). Samples isolated from cells subjected to the chemerin alone exhibited even higher level of Bcl-3 (Fig. 5 F line 3, Fig. 5 A). And again, as in the case of p50, nuclear abundance of Bcl-3 dropped in the cell cultures incubated with combination of caerulein and chemerin (Fig. 5 F line 4, Fig. 5 H) reaching almost level observed in the control cells (Fig. 5 F line 1, Fig. 5 H).

Discussion

This study shows for the first time that application of chemerin to the rats with acute caerulein-induced pancreatitis resulted in the attenuation of inflammatory process in this gland. The mechanisms responsible for the reduction of acute pancreatitis at the cellular level are related, at least in part, to the inhibition of NF-κB with subsequent decrease of TNFα production.

Chemerin has been previously described as an proinflammatory adipokine, because its serum level was increased in chronic inflammatory diseases and correlated with inflammatory markers such as CRP and proinflammatory cytokines [11,15–17]. This

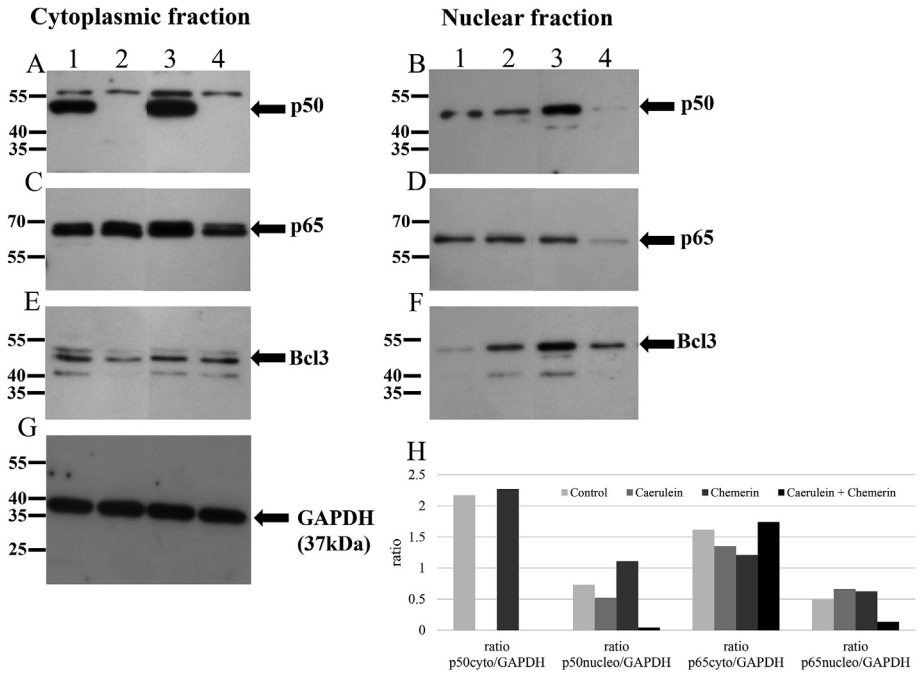

Fig. 5. Immunoblotting analysis of NF-κB components p50 (panel A), p65 (panel C) and Bcl-3 (panel E) cytoplasmic and nuclear (panels B, D, F) abundance in AR42J cells. Protein samples (5 μg per line) were probed with antibody for p50, p65 and Bcl-3 respectively. Line 1 – control cells, line 2 – cells incubated with caerulein (10 nM), line 3 – cells subjected to chemerin (100 nM) and line 4 – cells exposed to both factors caerulein (10 nM) and chemerin (100 nM). Blots shown in the figure were composed of two parts of the same exposure to remove unpresented data. Presented results were obtained in at least 3 consecutive experiments. The most representative results were picked for presentation. Panel G represents the analysis of cytoplasmic abundance of GAPDH to assess the equal load of samples on the lines. Panel H represents semi-quantitative analysis of the western-blot results for p50 and p65 proteins presented above. Values were expressed as the ratio of the optical density of the tested protein band to the optical density value of the corresponding GAPDH band.

adipokine was also detected in inflammatory exudates and in synovial fluid of patients with rheumatoid arthritis [17]. The pro-inflammatory effect of chemerin was supported by identification of its receptors on the immune cells and triggering by this adipokine the migration of macrophages toward the inflammatory site [11].

On the other hand chemerin was proposed to play a role in the resolution of inflammation. This protein could be subjected to proteolytic cleavage by serine- or cysteine proteases released from macrophages to create chemerin-derived peptides. These products of chemerin digestion reduced expression of inflammatory mediators on macrophages, inhibited macrophage activation and induced expression of anti-inflammatory IL-10 [12,26]. In mouse model of acute lung injury chemerin was shown to suppress the release of inflammatory cytokines [27]. However *in vitro* study did not clarify the molecular mechanism of this phenomenon [28]. Yamawaki et al. experiments have evidenced that chemerin is able to inhibit phosphorylation of pro-inflammatory NF-κB, and to reduce monocyte adhesion to the endothelial cells via inhibition of vascular cell adhesion molecule-1. This anti-inflammatory effect of chemerin was associated with stimulation of NO production [29].

Our present study is in agreement with described above observations concerning the anti-inflammatory properties of chemerin. Herein we demonstrate that this adipokine given to the rat with caerulein-induced acute pancreatitis significantly reduced serum amylase activity and TNFα level and diminish morphological signs of pancreatitis. Additionally, we have shown that chemerin ameliorated the signal of pro-inflammatory cytokine, TNFα, in the AR42J cells and this could be probably due to the modulation of NF-κB action. Results of *in vitro* experiments strongly supported the observed *in vivo* phenomenon of the decrease the serum level of TNFα in AP rats pretreated with chemerin.

Considering the fact that mild lysis conditions did not dissociate the protein complexes from the DNA which is precipitated during the isolation, the entire nuclear pool contains mostly unbound to DNA proteins and complexes. The strongly elevated nuclear translocation of p50 in response to the caerulein stimulation and formation of p50/p65 heterodimer resulted in complete depletion of the cytoplasmic pool from p50 (Fig. 5 A line 2). This suggests that p50 might play a role of limiting regulatory factor of this process and finds support in the strong activation of TNFα expression (Fig. 4 line 2), which is well known to be activated by NF-κB complexes. Chemerin alone did not change p50 cytoplasmic pattern when compared to the control (Fig. 5 A lines 1 and 3) but increased nuclear retention of p50, presumably as a p50/p50/Bcl-3 homodimer complex (Fig. 5 B lines 1 and 3). This was authenticated by the observation of very high level of Bcl-3 in the nuclear fraction after stimulation of the cells with chemerin alone (Fig. 3 F line 3). Similar observation was reported by Driessler K. et al. [30], who proposed anti-inflammatory mechanism utilized by IL-10 employing selective induction of nuclear translocation followed by DNA-binding of the repressive p50/p50 homodimer and in consequence attenuation of inflammatory related genes expression due to displacement of p50/p65 complexes from the promoters region. Involvement of Bcl-3 in generation of NF-κB homodimers in the cytoplasmic pool of p50 and their nuclear translocation has been well documented by Watanabe N. et al. [31] and more recently in Collins P. et al. [32], who demonstrated that interaction of Bcl-3 with p50 is necessary for the anti-inflammatory properties of NF-κB1–p50/p50/Bcl-3 complex. Our results pointed out the possibility of such interplay between NF-κB and NF-κB1 complexes in response to chemerin treatment of AR42J cell cultures.

Dynamics of TNFα and p50 protein level changes observed in the samples isolated from the cells being stimulated with both

caerulein and chemerin suggested that p50/p50/Bcl 3 complex might be involved in this anti-inflammatory reaction. The engagement of Bcl-3 in reduction of acute pancreatitis was also confirmed recently by Song L. et al. [33], who found elevated level of Bcl-3 in pancreatic tissue from mice and patients during course of acute pancreatitis. Authors proposed mechanism involving Bcl-3 in blocking ubiquitination and proteasome-mediated degradation of p50 homodimers. This observation stays in line with our result showing high nuclear level of p50 and Bcl-3 in response to caerulein or to chemerin (Fig. 5 B, F, lines 2 and 3). Moreover, as shown by Carmody et al. [34] Bcl-3 might regulate stability of p50 homodimers preventing from complex degradation and in this manner diminishing the extent of pancreatitis.

The most interesting observation is the depletion of nuclear abundance of p50, p65 and Bcl-3 in the samples collected from the cells incubated in the presence of both caerulein and chemerin (Fig. 5 B, D, F line 4). In our opinion the best explanation of this phenomenon is the displacement of p50/p65 complexes from the DNA binding sites, which then undergo proteolytic degradation and are therefore not detected. Non-canonical complex p50/p50/Bcl-3 is responsible for p50/65 displacement from the promoter region of TNFα gene, but as bound to DNA is not isolated under mild lysis conditions. The role of p50/p50/Bcl-3 in resolution of the inflammation via displacement of NFκB from promoters of pro-inflammatory genes finds the support in the experiments of Altavilla D. et al. [35], where authors proved that p50 deficient mice were not able to resolve the sterile, caerulein induced pancreatitis.

To sum up, we can propose a molecular model describing the modulatory role of chemerin during inflammatory response in AP (Fig. 6). In this model, under control condition we can expect a basic level of TNFα gene expression due to its weak activation with NFκB (p50/p65) complex. AP induced by caerulein increases TNFα gene expression possibly due to prolongation of the promoter up-regulation with NF-κB (p50/p65). Chemerin alone is not able to down-regulate even the basal TNFα gene expression but leads to the nuclear accumulation of homodimeric p50/p50-Bcl-3 complexes. Finally, under the pro-inflammatory condition caused by caerulein, chemerin is responsible for the displacement of the NF-κB complex (p50/p65) from its binding sites in the promoter of TNFα gene by replacing them with NF-κB1 p50/p50/Bcl-3 complexes inhibiting TNFα expression. Subsequently, NF-κB complex (p50/p65) may be directed to proteolytic degradation.

Concluding, chemerin significantly alleviated acute pancreatitis in the rat. This may be due to engagement of p50/p50/Bcl-3 to interfere and attenuate pro-inflammatory NF-κB signal transduction pathway in the pancreatic cells.

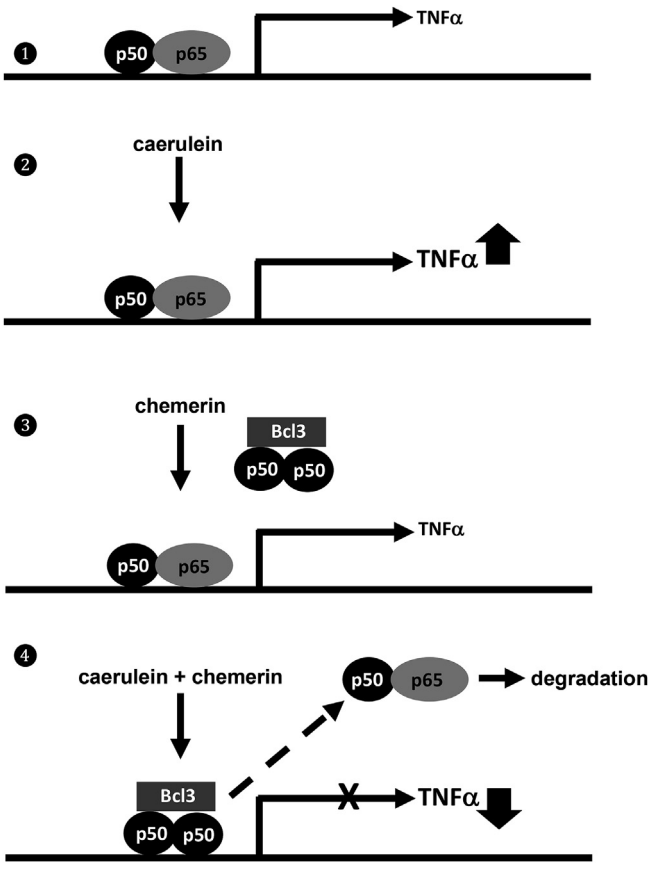

Fig. 6. The diagram illustrating the proposed mechanism of anti-inflammatory action of chemerin via activation of non-canonical homodimeric NF-κB1 (p50/p50) with Bcl-3 complexes and modulation of NF-κB binding. 1 – control condition with low/basic level of TNFα gene expression due to its activation with N-κB (p50/p65) complex; 2 – acute pancreatitis induced with caerulein increases TNFα gene expression possibly due to prolongation of the promoter up-regulation with NF-κB (p50/p65); 3 – chemerin alone is not able to down-regulate even the basal TNFα gene expression but leads to the nuclear accumulation of non-canonical homodimeric NF-κB1 (p50/p50) and Bcl-3 complexes; 4 – under the pro-inflammatory condition caused by caerulein, chemerin is responsible for the displacement of the NF-κB complex (p50/p65) from its binding sites in the promoter of TNFα gene by replacing them with NF-κB1 p50/p50/Bcl-3 complexes inhibiting TNFα expression. NF-κB complex (p50/p65) is directed to proteolytic degradation.

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