Pioglitazone, a PPAR-γ activator, attenuates the severity of cerulein-induced acute pancreatitis by modulating early growth response-1 transcription factor

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The purpose of this study was to test the hypothesis that activation of endogenous peroxisome proliferator-activated receptor (PPARγ) inhibits induction of early growth response factor-1 (Egr-1), which is rapidly induced in the pancreas following cerulein intraperitoneal injection. Acute pancreatitis was induced in mice by hourly intraperitoneal injection of cerulein. Pioglitazone was administered prophylactically and pancreatic inflammation was assessed. AR42J cells were stimulated with caerulein10^{-8}M co-incubated in presence of different concentration of pioglitazone. The expression of PPARγ, Egr-1, and the target genes of Egr-1 were studied by real-time reverse transcriptase polymerase chain reaction (PCR), Western blot, and immunohistochemistry. *In vitro*, a PPAR-γ activator (pioglitazone) strikingly diminished Egr-1 mRNA and protein expression corresponding to Egr-1. *In vivo*, treatment with pioglitazone prior to the intraperitoneal injection of cerulein induction of Egr-1 and its target genes such as, monocyte chemotactic protein-1 (MCP-1) and macrophage inflammatory protein-1 (MIP-1). The inhibitory effect of pioglitazone on Egr-1 expression induced by cerulein was almost fully restored by GW9662. Activation of PPAR-γ suppressed the activation of Egr-1 and its inflammatory gene targets and provided potent protection against pancreas injury. These data suggest a new mechanism in which PPAR-γ activation may decrease tissue inflammation in response to a cerulein insult. (Translational Research 2012;160:153–161)

Abbreviations: CBP/p300 = CREB-binding protein special SP-1 protein-1; DMSO = dimethylsulfoxide; Egr-1 = early growth response factor-1; IL-1 = interleukin-1; MCP-1 = monocyte chemotactic protein-1; MIP-1 = macrophage inflammatory protein-1; NF-κB = nuclear factor-κB; PCR = polymerase chain reaction; PPARγ = peroxisome proliferator-activated receptor; TZDs = thiazolidinedione derivatives

Acute pancreatitis is a common disease. In severe cases, mortality can reach up to 25% to 40%.[1] Cerulein pancreatitis is similar to human edematous pancreatitis involving dysregulation of the production of digestive enzymes and cytoplasmic vacuolization, the death of acinar cells, edema formation, and an infiltration of inflammatory cells into the pancreas. The activation of pancreatic enzymes in acinar cells and the digestion of pancreatic tissues have been generally regarded as the trigger for cerulean

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AT A GLANCE COMMENTARY

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Background

Egr-1 plays a major role in the immune and inflammation responses of pancreatitis. Recently, there is increasing evidence that the expression and activity of PPAR-γ may participate in the activity of Egr-1.

Translation Significance

Therefore, we investigated a putative relationship of the 2 transcription factors in experimental acute pancreatitis. Our findings define Egr-1 as novel targets of PPAR-γ and suggest that inhibition of Egr-1 gene transcription may be one of the mechanisms by which PPAR-γ regulates inflammatory response in experimental acute pancreatitis.

pancreatitis. Recent studies involving the transcription factor nuclear factor-κB (NF-κB) and other cytokines showed that inflammatory cytokines play an important role in the pathogenesis of acute pancreatitis due to their inflammation-inducing properties.[2]

Early growth response factor-1 (Egr-1), also referred to as nerve growth factor-induced protein A, Krox-24, or Zif268, is a transcription factor that may be an initial factor of inflammation.[3] The peroxisome proliferator-activated receptor (PPAR-γ) has recently been evaluated as a potentially important transcription factor. PPAR-γ modulates the inflammatory response of monocytes, which may underlie some of the anti-inflammatory effects of salicylates in rheumatoid arthritis.[4] PPAR-γ ligands inhibited the production of nitric oxide and macrophage-derived cytokines including tumor necrosis factor, interleukin-1 (IL-1), and IL-6 at least in part by antagonizing the activation of transcription factors such as NF-κB.[5,6] PPAR-γ, a member of the nuclear hormone receptor superfamily, was originally reported to be highly expressed in adipocytes and to play a critical role in their differentiation.[7] It is activated by the natural ligand 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15D-PGJ_2) as well as the synthetic ligand thiazolidinedione derivatives (TZDs).

The PPAR-γ gene is expressed in acinar cells and may respond to cerulein with an exuberant Egr-1-dependent inflammatory response.[8] Therefore, we hypothesized that physiologically, PPAR-γ expression may serve as an endogenous mechanism to dampen the pathologic response to cerulein triggered by Egr-1 induction. Experiments were undertaken to determine whether endogenous PPAR-γ and its activator TZD pioglitazone modulate the induction of Egr-1 and its inflammatory gene targets in response to cerulein.

MATERIALS AND METHODS

Cell culture. The first set of experiments was designed to determine if PPAR-γ activators could inhibit the increased expression of Egr-1 observed in AR42J cells. Rat pancreatic acinar AR42J cells were obtained from the American Type Culture Collection and cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma, St Louis, Mo) supplemented with 10% fetal bovine serum and antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin) with 44 mM sodium bicarbonate and 10% CO_2 as recommended.[9] The acinar cells were plated at a density of 2×10^6/mL in 6-well culture plates and allowed to attach for 12 h. The cells were pretreated with pioglitazone that was diluted in dimethylsulfoxide (DMSO), at a concentration of 0, 5, 10, 20, or 40 μM and 40 μM respectively. The last well was co-treated with the PPAR-γ antagonist GW9662 10 μM. Cells were then treated with cerulein (Sigma, St. Louis, Mo) at a concentration of 10^{-8} M half an hour later for half an hour.[10]

Experimental model of pancreatitis. Studies were performed on male Sprague Dawley (SD) rats weighing 200 to 250 g in accordance with the experimental protocol approved by the Committee for Research and Animal Ethics of China. Prior to the start of the experiments, rats were deprived of food but water was available ad libitum. Acute pancreatitis was induced by 50 μg/kg cerulein diluted in saline and injected intraperitoneally 2 times, 1 h apart as described previously.[8]

To study the effect of pioglitazone on the development of acute pancreatitis, the rats were divided into 6 groups (each with n = 10). The first was the sham plus saline group (ie, rats treated with 0.9% vehicle saline i.p and pretreated with DMSO, also referred to as the control group), and the second was rats treated with cerulein (50 μg/kg) i.p. hourly (×2) and pretreated with pioglitazone (40 mg/kg i.v. 30 min prior to cerulein infusion). The third group of rats were treated with cerulein (50 μg/kg) i.p. hourly (×2) and pretreated with pioglitazone (20 mg/kg i.v. 30 min prior to cerulein infusion) while the fourth group was treated with cerulein (50 μg/kg) i.p. hourly (×2) and pretreated with pioglitazone (10 mg/kg i.v. 30 min prior to cerulein infusion). The final 2 groups included rats treated with cerulein plus vehicle and rats treated with cerulein (50 μg/kg) i.p. hourly (×2) and pretreated with DMSO and rats treated with cerulein (50 μg/kg) i.p. hourly (×2) and pretreated with pioglitazone (40 mg/kg) and the PPAR-γ antagonist GW9662 0.3 mg/kg in the same time.