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Oxidative stress and inflammation in the pathogenesis of activated polyamine catabolism-induced acute pancreatitis

M. Merentie[1], **A. Uimari**[1], **M. Pietilä**[1], **R. Sinervirta**[1], **T. A. Keinänen**[1], **J. Vepsäläinen**[2], **A. Khomutov**[3], **N. Grigorenko**[3], **K.-H. Herzig**[1,4], **J. Jänne**[1], and **L. Alhonen**[1]

[1] Department of Biotechnology and Molecular Medicine, A. I. Virtanen Institute for Molecular Sciences, University of Kuopio, Kuopio, Finland
[2] Department of Chemistry, University of Kuopio, Kuopio, Finland
[3] Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia
[4] Department of Internal Medicine, Kuopio University Hospital, Kuopio, Finland

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Summary. The markers of oxidative stress and inflammation were studied in acute pancreatitis in transgenic rats exhibiting activated polyamine catabolism. In addition, the effect of bismethylspermine (Me_2Spm) pretreatment, preventing pancreatitis in this model, on these mediators was investigated. Lipid peroxidation was increased at 6 and 24 h after induction of pancreatitis. These changes as well as the markedly decreased superoxide dismutase activity at 24 h were abolished by Me_2Spm pretreatment. Glutathione level and catalase activity changed transiently, and the effect of Me_2Spm was clear at 24 h. Serum inflammatory cytokine levels increased already at 4 h whereas NF-κB was distinctly activated only at 24 h. Me_2Spm prevented the increase in TNF-α and IL-6 while it had no effect on NF-κB activation. These results show that typical inflammatory and, to a lesser degree, some oxidative stress mediators are involved and beneficially affected by the disease-ameliorating polyamine analogue in our pancreatitis model.

Keywords: Spermidine/spermine N^1-acetyltransferase – Inflammatory cytokines – Lipid peroxidation – Superoxide dismutase – Catalase – Glutathione

Abbreviations: AEBSF, 4-(2-aminoethyl)benzenesulfonyl fluoride; ELISA, enzyme-linked immunosorbent assay; EMSA, electrophoretic mobility shift assay; GSH, reduced glutathione; GSSH, oxidized glutathione; HRP, horse radish peroxidase; IκB-α, inhibitor-κBα; IL-1β, interleukin-1β IL-6, interleukin-6; IL-10, interleukin-10; LPO, lipid hydroperoxide; NO, nitric oxide; MDL72527, N^1,N^2-bis(2,3-butanediyl)-1,4-butanediamine; Me_2Spm, bismethylspermine; NF-κB, nuclear factor-κB; PAO, polyamine oxidase; PMSF, phenylmethanesulfonyl fluoride; SOD, superoxide dismutase; SSAT, spermidine/spermine-N^1-acetyltransferase; TNF-α, tumor necrosis factor-α

Introduction

Maintenance of polyamine homeostasis is essential for the functional and structural integrity of pancreas. Activation of polyamine catabolism in transgenic rats overexpressing spermidine/spermine N^1-acetyltransferase (SSAT) results in severe acute pancreatitis (Alhonen et al., 2000). The final outcome in this experimental disease model can be ameliorated with stable α-methylated polyamine analogues, which prevent the development of pancreatitis when given before the induction of SSAT (Räsänen et al., 2002) and dramatically improve survival of the animals when given after the induction of SSAT (Hyvönen et al., 2006). The exact mechanism of polyamine catabolism-induced pancreatitis is not known but it appears to involve premature trypsinogen activation in vitro in cultured acini (Hyvönen et al., 2006) and in vivo in transgenic rats (Hyvönen et al., 2007).

Severe acute pancreatitis is a result of primary damage in acinar cells caused by trypsinogen activation, followed by local inflammatory reaction and later by systemic inflammatory response, and multiorgan dysfunction in the worst case. The type of pancreatic cell death (apoptotic versus necrotic) and the extent of systemic inflammation mediated by pro- and anti-inflammatory cytokines are the determinants of the severity of the disease (Bhatia et al., 2005). Oxidative stress is also considered as an important factor in the pathogenesis of acute pancreatitis, as free radical scavengers have been found to ameliorate the outcome of changes associated with pancreatitis in various models (reviewed by (Schulz et al., 1999). However, the role of reactive oxygen species as being crucial factors in

initiating pancreatitis in vivo has been questioned. In an approach to solve this question, Rau et al. demonstrated that in taurocholate-induced pancreatitis, extracellular generation of oxygen free radicals did not induce pancreatitis but that the radicals were important mediators of tissue damage (Rau et al., 2000). In a recent review by Pereda and coworkers, the interaction between pro-inflammatory cytokines and oxidative stress is defined as the major cause leading to amplification of uncontrolled inflammatory cascade and initiation of systemic inflammatory response (Pereda et al., 2006).

The triggering event in our model is the transgene activation-induced rapid acetylation of intracellular polyamines leading to severe polyamine depletion in few hours. The detachment of polyamines from their putative binding sites, such as membranes and other cellular structures, is assumed to allow trypsinogen activation to occur (Hyvönen et al., 2007). As polyamines have been shown to possess anti-inflammatory (Hasko et al., 2000; Perez-Cano et al., 2003; Seiler and Atanassov, 1994; Zhang et al., 1997; Zhang et al., 2000) and antioxidant properties (Lovaas and Carlin, 1991; Matkovics et al., 1993), the present work was carried out to investigate the role of oxidative stress and inflammation in the development of pancreatitis induced by activated polyamine catabolism.

Materials and methods

Animals

Transgenic rats with metallothionein promoter-driven SSAT gene (Alhonen et al., 2000) and their syngenic littermates were used. Activation of the transgene was induced by an injection of 10 mg zinc/kg body weight i.p. as zinc sulfate (Merck). Me_2Spm was synthesized as described earlier (Grigorenko et al., 2005) and administered 20 and 4 h before zinc. The rats were anesthetized with CO_2, thoracotomized and blood was collected by puncturing the heart before decapitation. Pancreatic tissue was removed, processed immediately or frozen in liquid nitrogen and stored at $-70\,^{\circ}C$ until analyses. The Institutional Animal Care and Use Committee of the University of Kuopio and the Provincial Government approved the animal experiments.

Determination of pancreatic SSAT activity, polyamine levels and plasma α-amylase activities

SSAT and polyamines were determined by the published methods (Bernacki et al., 1995; Hyvönen et al., 1992). Plasma amylase activity was measured using the analyzer system Microlab 200 from Merck (Darmstadt).

Analyses of oxidative stress markers

Lipid hydroperoxides indicating lipid peroxidation and cytoplasmic superoxide dismutase (SOD) activity were measured using commercially available kits (Lipid Hydroperoxide (LPO) Assay Kit and Superoxide Dismutase Assay Kit, Cayman Chemicals, Ann Arbor, MI). The levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) were determined fluorometrically according to previously described method (Senft et al., 2000). Measurement of catalase activity was carried out according to Aebi (1984). The decomposition of H_2O_2 was followed by the decrease in absorbance at 240 nm. Catalase activity was defined as the change in the absorbance per minute per milligram tissue (wet weight). Serum nitric oxide (NO) concentration was measured with a commercial kit (Total Nitric Oxide Assay Kit, Endogen, Rockford, IL).

Preparation of nuclear and cytoplasmic extracts

Protein extraction was based on a published method (Dignam et al., 1983). Frozen tissue was powdered in a mortar in liquid nitrogen. The powdered sample (100 to 200 mg) was lysed in 1 ml of buffer A using a glass homogenizer. The buffer A (10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl) was supplemented with dithiothreitol (DTT) and phenylmethylsulfonyl fluoride (PMSF) to a final concentration of 1 mM each and with the protease inhibitor cocktail (Sigma, final concentrations: 1 mM AEBSF, 0.8 μM aprotinin, 21 μM leupeptin, 36 μM bestatin, 15 μM pepstatin A, 14 μM E-64). The homogenate was kept on ice for 20 min and then Nonidet P-40 was added to a final concentration of 0.3%. Nuclei were collected by microsentrifugation for 30 sec. The supernatant fraction (cytosolic proteins) was saved for Western blot analysis and the pellet was suspended into 120 μl of buffer C (20 mM HEPES, pH 7.6, 25% (v/v) glycerol 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 20 mM β-glycerophosphate, 10 mM Na_2MoO_4, 50 μM Na_2Vo_4) supplemented with DTT, PMSF and protease inhibitor cocktail as described above. Samples were mixed by rotating at $+4\,^{\circ}C$ for an hour, microsentrifuged for 10 min after which the supernatant (nuclear extract) was collected, aliquoted and stored at $-80\,^{\circ}C$. Protein concentrations in the extracts were determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA).

Electrophoretic mobility shift assay (EMSA) for nuclear factor (NF)-κB binding activity

Binding reactions and oligonucleotide labeling for the electrophoretic mobility shift assay were essentially performed by applying the Gel shift assay system (Promega) according to the manufacturer's instructions. Ten micrograms of nuclear extracts were mixed with the binding buffer (Promega) and incubated for 10 min at room temperature. ^{32}P-labeled DNA probe ($4–8 \times 10^5$ cpm) was added to the reaction (10 μl total volume) and incubated further for 20 min at room temperature. One microliter of 10× gel loading buffer (250 mM Tris-HCl, pH 7.5, 0.2% bromophenol blue, 40% glycerol) was added into each reaction and the samples were electrophoresed in 1× TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA) on nondenaturing 5% polyacrylamide gel at 250 V for 2 h. Gels were dried and exposed to a phosphor screen (Molecular Dynamics, Sunnyvale, CA). Reading of the screen was done with the Storm 860 scanner (Molecular Dynamics, Sunnyvale, CA). For cold competition 30 to 50× molar excess of nonlabeled oligonucleotide was added to the reaction with the probe. The subunit composition of the activated NF-κB was determined with supershift experiments, in which 2 μg of antibody against NF-κB proteins p65 or p50 (sc-7151, sc-114; Santa Cruz Biotechnology, Santa Cruz, CA) was added to the reaction 50 min before the addition of the probe.

Determination of the amount of inhibitor-κBα protein with Western blot analysis

Equal amounts of cytoplasmic protein extracts (50 μg) were resolved in 12% SDS polyacrylamide gels in Tris-Glycine-SDS buffer. Separated proteins were electrophoretically transferred to polyvinylidene fluoride membrane for 1 h at 100 V. Blots were blocked for 1 h in 5% (wt/vol)

nonfat dry milk in Phosphate buffered saline (PBS, pH 7.2) containing 0.1% (vol/vol) Tween 20, washed twice for 10 min with PBS-Tween 20 buffer mentioned above (washing buffer) and incubated for 1 h with 1:500 diluted IκB-α primary antibody (sc-371-G; Santa Cruz Biotechnology, Santa Cruz, CA) in blocking buffer. Blots were washed three times for 10 min with washing buffer and incubated with the HRP secondary antibody (sc-2020, Santa Cruz Biotechnology, Santa Cruz, CA) present at a dilution of 1:3000 in blocking buffer. Membranes were washed four times with the washing buffer and developed for visualization using Western lightning chemiluminescence Reagent Plus detection kit (Perkin Elmer Life Sciences, Inc., Boston, MA).

Measurement of cytokine concentrations

Serum levels of cytokines TNF-α, IL-1β, IL-6 and IL-10 were determined with ELISA kits according to manufacturer's instructions (Quantikine Rat TNF-α, IL-1β, IL-6 or IL-10 Immunoassay ELISA kit, R&D systems, Minneapolis, MN).

Statistical analyses

The data are expressed as means ± standard error of the mean (S.E.M.). One-way analysis of variance with Bonferroni's post hoc test for multiple comparisons was used in statistical analyses with GraphPad Prism 4.0 (GraphPad Software, Inc., San Diego, CA).

Results

Activation of polyamine catabolism and development of pancreatitis

Activating the metallothionein promoter-driven transgene, SSAT, with a non-toxic dose of zinc induced acute pancreatitis in the transgenic rats. As an indication of activated polyamine catabolism in response to zinc, pancreatic SSAT was strikingly increased at 6 and 24 h after administration of zinc (Table 1). The enzyme activation was associated with accumulation of putrescine, the end product of polyamine catabolism, and profound depletion of spermidine and spermine concentrations. While further increasing SSAT activity, pretreatment with Me_2Spm had virtually no effect on the depleted pools of the natural polyamines but efficient accumulation of the SSAT-resistant analogue was observed (Table 1).

Increase in the level of α-amylase activity in circulation is one of the characteristics of acute pancreatitis. α-Amylase remained normal at 6 h but was significantly elevated at 24 h after zinc (Table 1). This elevation was partially prevented by the pretreatment with Me_2Spm (Table 1).

These results are similar to those reported earlier and it should be pointed out that, in addition to elevated amylase activities, the zinc-treated animals exhibit extensively necrotic pancreatic tissue at 24 h whereas the tissue in the animals pretreated with the polyamine analogue is protected from damage (Alhonen et al., 2000; Räsänen et al., 2002). It is also noteworthy that the first signs of developing pancreatitis, such as trypsinogen activation, ultrastructural cellular changes, edema, inflammation and occasional necrosis, were seen already at 4 h after induction in zinc-treated animals (Hyvönen et al., 2006, 2007).

Oxidative stress

Induction of acute pancreatitis with zinc resulted in increased amount of lipid hydroperoxides, products of lipid peroxidation, at 6 and 24 h. Administration of Me_2Spm prior to induction of pancreatitis attenuated the lipid peroxidation at both time points, as depicted in Fig. 1A. Activity of cytoplasmic SOD was only slightly decreased at 6 h but significantly decreased ($p < 0.01$) at 24 h in zinc-treated rats in comparison with the control animals. In rats pretreated with Me_2Spm, the decrease was largely prevented, as SOD activity was almost comparable to SOD activity in the control group (Fig. 1B). In contrast, the changes in catalase activity and reduced glutathione, GSH, level in response to zinc were not that clearly in line. Catalase activities were decreased at 6 h but increased at 24 h above the control level and even further in the group treated with Me_2Spm (Fig. 1C). GSH level was somewhat elevated at

Table 1. Pancreatic SSAT activities, polyamine concentrations and amylase activities in transgenic rats after treatment with zinc and Me_2Spm

Group	SSAT pmol/mg/10 min	Putrescine pmol/mg tissue	Spermidine pmol/mg tissue	Spermine pmol/mg tissue	Me_2Spm pmol/mg tissue	α-Amylase U/l
Control (3)	20 ± 7	1390 ± 510	5040 ± 290	557 ± 19	NA	3140 ± 340
Zn 6 h (6)	1090 ± 86	4140 ± 240***	830 ± 50***	338 ± 20***	NA	2680 ± 330
Zn 6 h + Me_2Spm (5)	4930 ± 770	4390 ± 91***	560 ± 36***	233 ± 24***	908 ± 100	3030 ± 590
Zn 24 h (5)	6390 ± 1730*	3340 ± 175***	270 ± 56***	105 ± 19***	NA	21740 ± 3610**
Zn 24 h + Me_2Spm (6)	8620 ± 1290***	3940 ± 185***	360 ± 78***	91 ± 8***	538 ± 55	12490 ± 3240

The rats received zinc (10 mg/kg i.p.) without or with Me_2Spm (25 mg/kg i.p.) administered 20 and 4 h before zinc. The animals were sacrificed 6 or 24 h after zinc administration. The data are expressed as means ± S.E.M. The number of rats per group is given in parentheses. *NA* Not applicable. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ as compared with untreated control animals

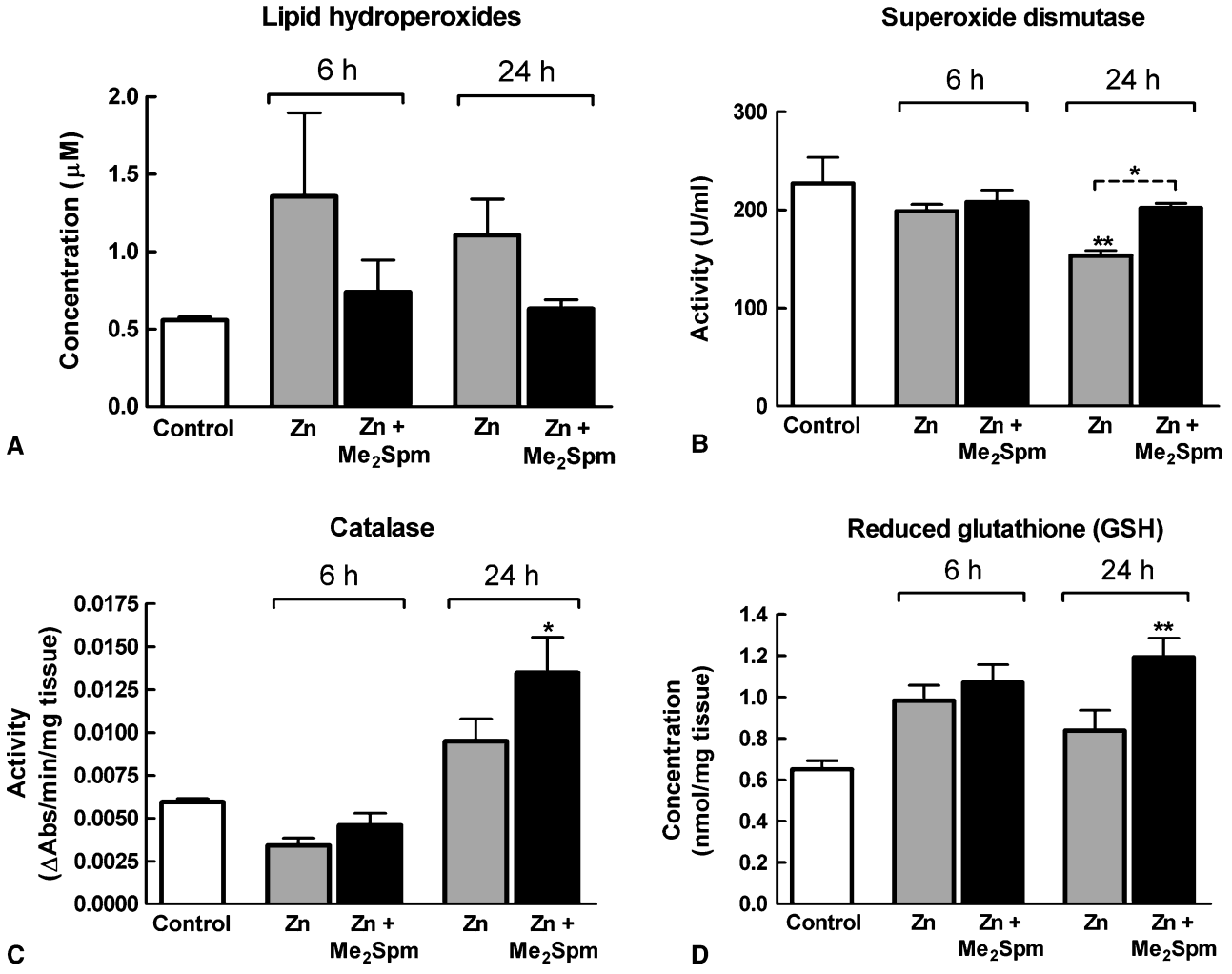

Fig. 1. Effect of zinc administration and Me_2Spm pretreatment on oxidative stress markers. Lipid hydroperoxides (**A**), activities of SOD (**B**) and catalase (**C**) and levels of reduced GSH (**D**) were determined at 6 and 24 h after induction of acute pancreatitis in the transgenic rats. The data are expressed as means ± S.E.M. $^{*}p<0.05$, $^{**}p<0.01$ as compared with untreated animals. Significance in the difference between zinc-treated and Me_2Spm pretreated groups is indicated above the dotted line

both time points studied, and Me_2Spm pretreatment resulted in no change at 6 h but in an even greater increase in GSH level at 24 h (Fig. 1D). There were virtually no changes in the level of oxidized glutathione, GSSG, in any group (results not shown).

In a separate experiment serum level of NO was determined at 12, 24 and 36 h after zinc administration with or without Me_2Spm treatment given 4 and 8 h after zinc. There was a slight rise from $8.2 \pm 0.8\,\mu M$ to $15.8 \pm 13.1\,\mu M$ at 12 h in the concentration of serum NO on which Me_2Spm treatment had no significant effect ($21.0 \pm 4.5\,\mu M$). Serum NO was markedly elevated at 24 h ($48.1 \pm 14.4\,\mu M$; $p<0.05$ as compared with the control group) and treatment with Me_2Spm slightly decreased its concentration (40.5 ± 15.1). The corresponding values at 36 h were $73.6 \pm 2.3\,\mu M$ ($p<0.001$) in zinc-treated group and $66.3 \pm 18.2\,\mu M$ in the group treated with Me_2Spm and zinc.

Inflammatory factors

Activation of NF-κB was studied at several time points during the development of activated polyamine catabolism-induced pancreatitis. The earliest time point when the activation of NF-κB could be detected was 4 h after zinc albeit there were great variations among the animals within the group (not shown). More consistent NF-κB activation could be observed at time points from 12 h onwards after induction of acute pancreatitis (not shown). The activation of NF-κB was clearly evident at 24 h after zinc administration, as demonstrated by a representative EMSA shown in Fig. 2A, in which the samples from transgenic,

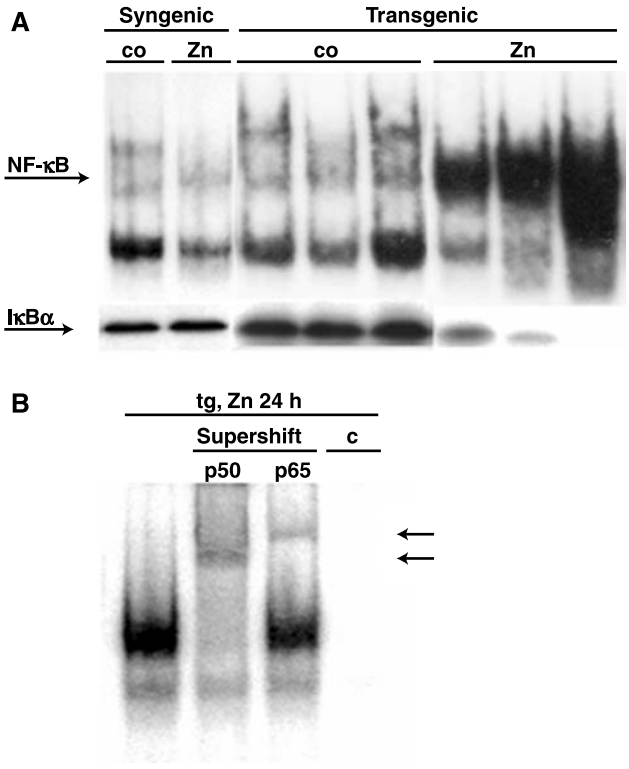

Fig. 2. Activation of pancreatic NF-κB in untreated (co) and zinc-treated (Zn) syngenic and transgenic animals. **A** DNA-binding activity of NF-κB detected by EMSA and the presence of cytoplasmic IκBα protein detected by Western blot analysis at 24 h after zinc administration. Arrows indicate NF-κB and IκBα-specific bands. **B** The binding specificity and subunit composition of NF-κB dimer activated in the polyamine catabolism induced acute pancreatitis. Pancreatic nuclear extracts of transgenic rats treated 24 h with zinc were subjected to EMSA either with ^{32}P-labeled NF-κB consensus oligonucleotide alone (first lane), with cold probe for competition reaction (marked with c) or with p50 or p65 antibodies for supershift reactions. The arrows indicate the upshifted bands

zinc-treated animals show substantial activation as compared with syngenic and transgenic control animals. At later time points beyond 24 h after zinc induction only few animals survived, and in them, an immense induction of pancreatic NF-κB activation could be seen (not shown). Zinc treatment did not cause activation of NF-κB in syngenic animals (Fig. 2A). In normal circumstances, NF-κB is kept inactive in the cytoplasm with the aid of IκB inhibitory proteins. Prior to NF-κB activation, e.g. during the inflammatory reactions, the bound IκB is phosphorylated and targeted to ubiquitination and degradation, thus releasing the transcription factor to translocate into the nucleus. The amount of inhibitor-κBα was clearly decreased in the samples with activated NF-κB and the amount correlated inversely with the NF-κB activation (Fig. 2A). The competition and supershift reactions, depicted in Fig. 2B, show that the NF-κB binding was specific and that the activated NF-κB complex contained both p50 and p65 proteins.

Pretreatment of rats with Me_2Spm before zinc induction of acute pancreatitis appeared to partially prevent NF-κB DNA binding activity at 24 h after zinc. The quantitated intensities of EMSA signals rose from control values of 1.5 ± 0.5 to 4.8 ± 1.0 in zinc-treated group whereas the intensity was 3.0 ± 0.9 in the Me_2Spm treated group at 24 h.

Figure 3 depicts the serum levels of cytokines TNF-α, IL-1β, IL-6 and IL-10 analyzed during the course of polyamine catabolism-induced acute pancreatitis. All the circulating cytokines were upregulated already in the earliest time point (4 h), although the kinetics of each cytokine were different. The levels of TNF-α (Fig. 3A) and IL-1β (Fig. 3B) were clearly elevated at 4 h and reached their highest point at 20 h, decreasing from that slightly at 24 h. Instead, the level of IL-6 (Fig. 3C) reached its highest value at 4 h after zinc treatment, after which it started to decline. Serum level of IL-10 (Fig. 3D) was significantly increased only after 12 h and stayed clearly upregulated up to 24 h after induction of acute pancreatitis.

The effect of Me_2Spm pretreatment on cytokines TNF-α and IL-6 was studied at 6 and 24 h after induction of acute pancreatitis. As shown in Fig. 4, the levels of both cytokines were greatly increased at both time points. Me_2Spm pretreatment significantly reduced the amount of TNF-α at 6 and 24 h after zinc, the effect being more pronounced at the later time point (Fig. 4A). Similarly, Me_2Spm treatment decreased the serum level of IL-6 at 24 h in contrast to 6 h, when Me_2Spm insignificantly increased the cytokine level (Fig. 4B).

Discussion

In transgenic rats with activated polyamine catabolism, acetylated polyamines are metabolized further in polyamine oxidase (PAO)-catalyzed reaction to yield hydrogen peroxide and reactive aldehydes potentially creating oxidative stress. We have earlier studied the role of these molecules in the pathogenesis of pancreatitis with the aid of a PAO inhibitor MDL72527. While inhibiting PAO activity, the treatment of animals with the combination of the inhibitor and zinc caused even more severe pancreatitis than zinc alone suggesting that the intracellularly generated products of polyamine oxidation are not triggers of and do not significantly contribute to the inflammatory process in our model (Alhonen et al., 2000). The present results, however, show that oxidative stress, as indicated by increased lipid peroxidation, is associated with

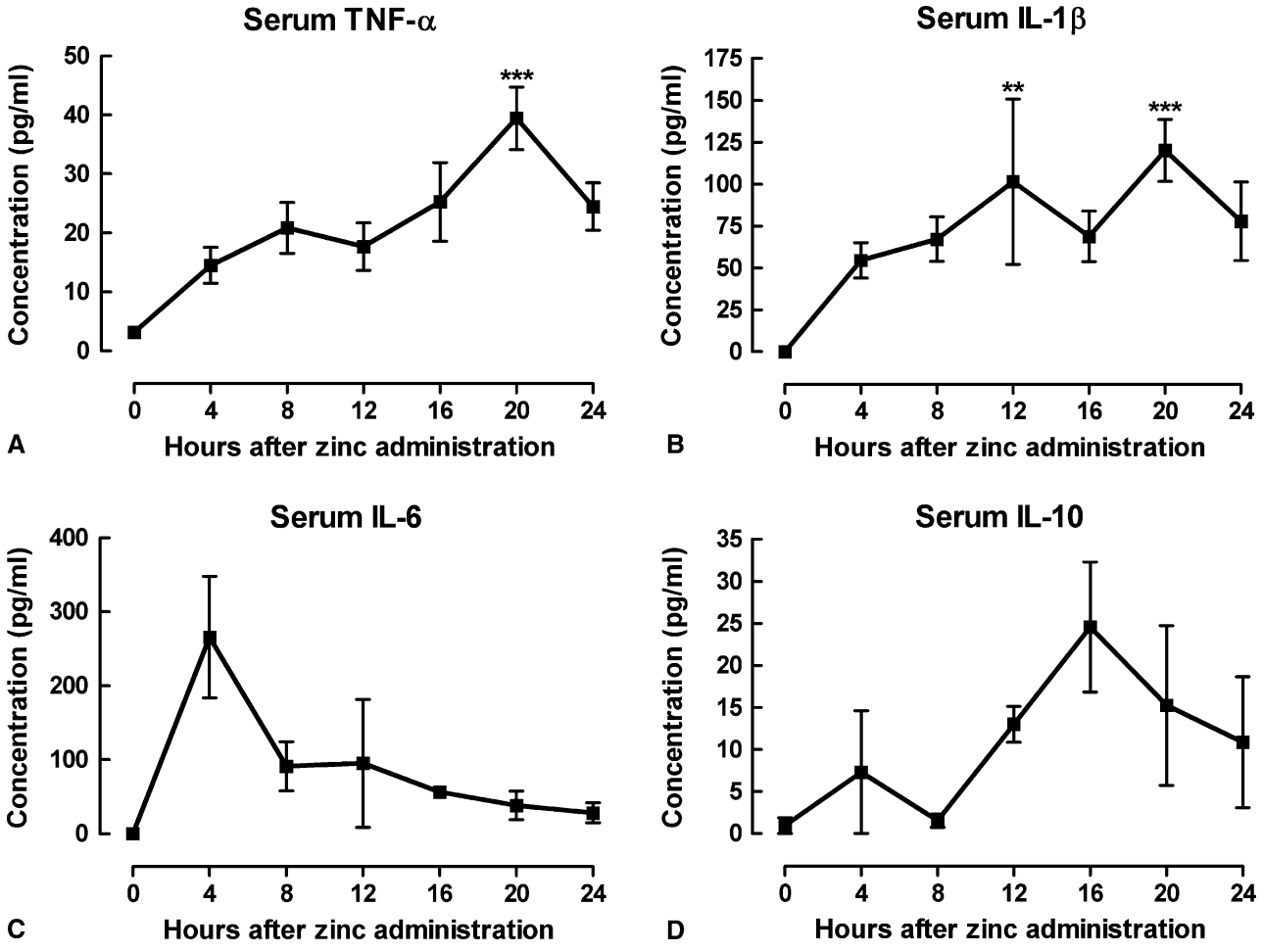

Fig. 3. Circulating levels of cytokines TNF-α (**A**), IL-1β (**B**), IL-6 (**C**) and IL-10 (**D**) in the course of polyamine catabolism-induced acute pancreatitis. The data are expressed as means ± SEM. $^{*}p < 0.05$ as compared with untreated (0 h) samples

the development of pancreatitis (Fig. 1). The fact that increased lipid peroxidation could be alleviated with a prior treatment with Me_2Spm leads to the hypothesis that depletion of polyamines from membrane structures exposes the membranes to lipid peroxidation caused by reactive oxygen species generated in acinar cells. This hypothesis is supported by a recent observation on the protective role of polyamines against membrane lipoperoxidation in Trypanosoma cruzi (Hernandez et al., 2006). It is also possible that diminished polyamine-mediated antioxidant effect resulting from activated polyamine catabolism may further facilitate the generation and accumulation of reactive oxygen species in acinar cells, e.g. through activation of xanthine oxidase (Sanfey et al., 1985).

As an impairment of primary defense, decrease in pancreatic antioxidant GSH and activities in free radical scavenging enzymes catalase and SOD are generally involved in acute pancreatitis. In our model, a marked decrease in SOD activity, preventable with Me_2Spm pretreatment was evident at 24 h after induction of the disease. However, GSH and catalase were not affected that consistently, as instead of decreasing, glutathione levels seemed to rise in the course of acute pancreatitis (Fig. 1). The transient decrease seen in catalase activity at 6 h after induction of pancreatitis was partially prevented by Me_2Spm treatment and fully reverted by 24 h even without the analogue treatment (Fig. 1). Upregulation of NO in the serum was also associated with the acute pancreatitis in our model rats but Me_2Spm had no clear effect on the circulating levels of NO. The role of NO in the pathogenesis of pancreatitis is somewhat controversial but it may be important in the systemic response. Interestingly, in our model, the level of NO increased with developing pancreatitis and the highest NO levels were seen at the late phases of the disease (36 h) when systemic changes associated with mortality are evident. As polyamines have been shown to inhibit induction of NO synthase (Szabó et al., 1994), it is also possible that the depletion of pancreatic

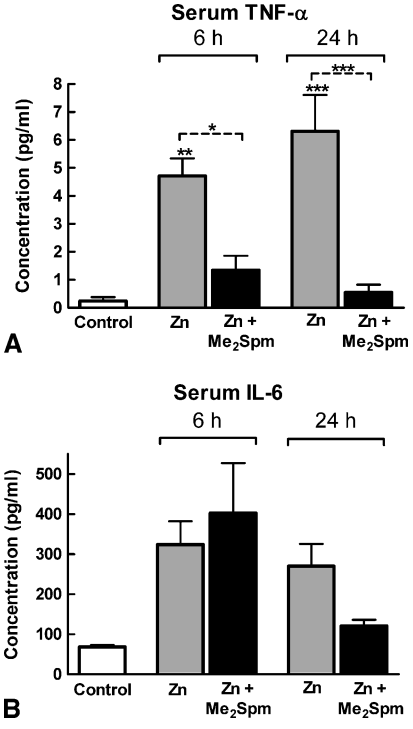

Fig. 4. The effect of Me_2Spm pretreatment on serum levels of cytokines TNF-α (**A**) and IL-6 (**B**). The cytokine levels were determined at 6 and 4 h after zinc administration. The values are means ± S.E.M. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared with the control animals. Significance in the differences between zinc-treated and Me_2Spm pretreated groups is indicated above with the dotted lines, $^{*}p<0.05$, $^{***}p<0.001$

polyamines at least partially contributes to the level of circulating NO by inducing the synthesis of NO.

The serum levels of the key pro-inflammatory cytokines TNF-α and IL-1β were increased during the first hours after induction of pancreatitis (Fig. 3). In the amplification of inflammatory process, these cytokines are believed to further induce their own expression as well as the expression of other cytokines in activated leukocytes (Norman, 1998). Serum levels of the pro-inflammatory IL-6 and anti-inflammatory IL-10 were upregulated already at 4 h and the latter reached its highest level at 16 h after induction. Keeping the severity of the disease in mind, the anti-inflammatory IL-10 cannot cope with the uncontrolled inflammatory cascade, enabling the vigorous progress of the disease. A regulatory signaling cascade involving transcription factor NF-κB is also known to be activated in acute pancreatitis but NF-κB apparently had little effect on the upregulation of proinflammatory cytokines at the early phases of pancreatitis in our model, as its activation in pancreas followed slower kinetics than the increases in the levels of the cytokines (Figs. 2 and 3). Vice versa, it is likely that the earlier increased levels of cytokines, e.g. TNF-α, regulate the activation of NF-κB and the subsequent signaling cascade (Garg and Aggarwal, 2002).

Assuming that the inflammatory response is initiated by acinar cell damage after trypsinogen activation, the beneficial effect of Me_2Spm pretreatment on many of the parameters measured here (lipid peroxidation, SOD, catalase, TNF-α IL-6) can be understood as a result of analogue-mediated protection from cellular damage. In most cases, the effect of Me_2Spm is more prominent in the later phases of the pancreatitis, i.e. at 24 h when systemic disease evolves in animals not treated with the analogue. The fact that some mediators, such as NO, NF-κB and IL-6 were only partially affected by Me_2Spm suggests that secondary mechanisms unrelated to polyamine depletion or mechanisms in which the analogue is unable to compensate for the loss of the natural polyamines are involved.

The present results show that the development of pancreatitis in SSAT transgenic model involves activation of mediators associated with inflammation and oxidative stress. Based on the less profound changes in response to zinc, the role of oxidative stress may be of minor importance than that of inflammatory factors in the early stages of acute pancreatitis in this model. However, the crosstalk between these mediators determines the final outcome of the disease as regards severity (Pereda et al., 2006).

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Authors' address: Leena Alhonen, A. I. Virtanen Institute for Molecular Sciences, University of Kuopio, P.O. Box 1627, FI-70211 Kuopio, Finland, Fax: +358 17 163025, E-mail: Leena.Alhonen@uku.fi