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Short communication

Inhibition of lipopolysaccharide/ATP-induced release of interleukin-18 by KN-62 and glyburide

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Abstract

Monocytes release interleukin-18 after activation by lipopolysaccharide/ATP. Since inflammatory conditions such as sepsis are characterized by augmented interleukin-18 in sera of patients, we sought to modulate lipopolysaccharide/ATP-induced interleukin-18 release by pharmacological means. Here we report that 1-[*N*,*O*-bis(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine (KN-62), an inhibitor of ATP-mediated cellular activation by the purinoreceptor subtype P_{2x7}, potently suppresses interleukin-18 release from peripheral blood mononuclear cells. Interleukin-18 liberation was likewise inhibited by glyburide, a modulator of ion transport and inhibitor of ATP-binding cassette transporter 1. The data presented herein indicate that by pharmacologically interfering with the process of cytokine secretion agents such as KN-62 or glyburide have the potential to curb overproduction of interleukin-18 in septic patients.

Keywords: Interleukin-18; Lipopolysaccharide; Monocyte; Inflammation; Glyburide; Purinoreceptor subtype P_{2x7}

1. Introduction

Interleukin-18 is a pro-inflammatory member of the interleukin-1 family of cytokines, shares several biological properties with interleukin-1β, and is a pivotal mediator of interferon-γ production (Dinarello, 1996; Gracie et al., 2003). However, in contrast to interleukin-1β and tumor necrosis factor-α, interleukin-18 is constitutively expressed in a variety of cell types among them peripheral blood mononuclear cells (Puren et al., 1999). This characteristic together with the ability of interleukin-18 to mediate production of tumor necrosis factor-α and interleukin-1β (Puren et al., 1998) suggests that interleukin-18 is located at a rather proximal position in the pro-inflammatory cytokine cascade. Therefore, blockage of interleukin-18 bioactivity has the potential to become a key component of anti-cytokine strategies (Dinarello, 2000). In fact, enhanced levels of interleukin-18 have been associated with human diseases such as rheumatoid arthritis (Yamamura et al., 2001), type 1 diabetes (Nicoletti et al., 2001a), multiple sclerosis (Nicoletti et al., 2001b), myasthenia gravis (Jander and Stoll, 2002), Crohn's disease (Pizarro et al., 1999), and septic shock syndrome (Grobmyer et al., 2000). Suppression of interleukin-18 bioactivity is protective in the respective animal models of these immunoinflammatory/autoimmune diseases (Plater-Zyberk et al., 2001; Nicoletti et al., 2003; Wildbaum et al., 1998; Im et al., 2001; Siegmund et al., 2001; Ten Hove et al., 2001; Netea et al., 2000). Accordingly, recent reports demonstrate the anti-inflammatory potential of interleukin-18 blockage by use of interleukin-18 binding protein in human whole blood cultures (Stuyt et al., 2001; Nold et al., 2003).

Previous studies suggest that release of interleukin-18 from human monocytes is a rather inefficient process. Actually, induction of interleukin-18 secretion was not detected in peripheral blood mononuclear cells exposed to lipopolysaccharide (Puren et al., 1999). In contrast, induction of release of pro- and mature interleukin-18 from monocytes can be achieved by incubation with the combination lipopolysaccharide plus ATP (3 mM). This process is mediated by binding of ATP to the purinoreceptor subtype P_{2x7} and associated with caspase-1 activation (Metha et al., 2001). Accordingly, lipopolysaccharide/ATP-induced secretion of interleukin-1β ex vivo is completely suppressed in whole

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blood cultures obtained from P_{2x7} knockout mice (Labasi et al., 2002). Prolonged activation of P_{2x7} triggers pore formation and cell death in monocytic cells (Di Virgilio et al., 1998). Cellular mechanisms that initiate release of interleukin-1β from monocytes may likewise apply to interleukin-18. Therefore, effects of two well-characterized inhibitors of lipopolysaccharide/ATP-induced interleukin-1β release (Hamon et al., 1997; Grahames et al., 1999) were investigated with regard to their effects on interleukin-18 secretion. Specifically, we analyzed the modulatory potential of 1-[*N*,*O*-bis(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine (KN-62), a potent antagonist of P_{2x7} activation (Humphreys et al., 1998; Grahames et al., 1999; Eschke et al., 2002). Since IL-18, like IL-1β, lacks a signal peptide (Dinarello, 1996; Gracie et al., 2003) and its secretion may thus involve the action of ATP-binding cassette (ABC) transporters, we also investigated effects of glyburide, a potassium channel blocker and inhibitor of ABC transporter 1 (Hamon et al., 1997).

2. Materials and methods

Lipopolysaccharide (*E. coli* serotype 0127:B8) and ATP were from Sigma (Deisenhofen, Germany). Glyburide and KN-62 were from Calbiochem (Schwalbach, Germany). The study protocol and consent documents were approved by the Ethik Kommission of the Klinikum der Johann Wolfgang Goethe-Universität, Frankfurt am Main. Healthy volunteers abstained from using any drugs during 2 weeks before the study. Peripheral blood mononuclear cells were freshly isolated from heparanized blood as described and incubated at the indicated cell-density in RPMI 1640 supplemented with 25 mM HEPES, 100 U/ml penicillin, 100 μg/ml

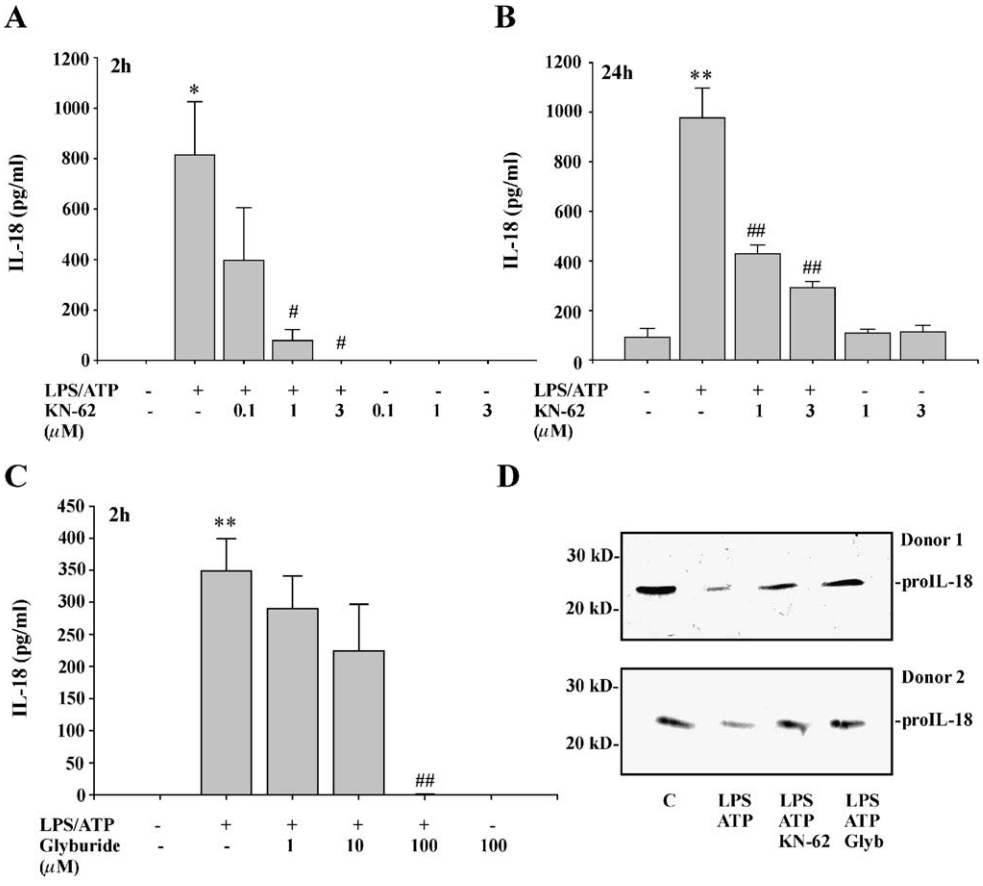

Fig. 1. KN-62 and Glyburide inhibit lipopolysaccharide/ATP-induced interleukin-18 release from peripheral blood mononuclear cells. Human peripheral blood mononuclear cells were seeded at 5×10^6 cells/ml (ABD) or at 3×10^6 cells/ml (C) and stimulated with the combination lipopolysaccharide (100 ng/ml)/ATP (3 mM) for either 2 h (ACD) or 24 h (B). Peripheral blood mononuclear cells were coincubated with either KN-62 or glyburide. KN-62 or glyburide were either used at the indicated concentrations (ABC), or with KN-62 at 3 μM or glyburide at 100 μM (D). Glyburide (2 h) and KN-62 (10 min) were applied before the addition of lipopolysaccharide/ATP. IL-18 concentrations in cell-free supernatants were determined by ELISA. Data are expressed as mean interleukin-18 concentrations ± S.E.M. ($n=3$ for (AC) or $n=4$ for (B)). *$p<0.05$, **$p<0.01$ compared to untreated control; #$p<0.05$, ##$p<0.01$ compared to peripheral blood mononuclear cells treated with lipopolysaccharide/ATP alone. Vehicle control (0.06% DMSO) did not significantly affect interleukin-18 release in response to lipopolysaccharide/ATP (348.9 ± 50.6 pg/ml versus 394.8 ± 62.8 pg/ml for lipopolysaccharide/ATP versus lipopolysaccharide/ATP (DMSO at 0.06%), 2 h stimulation, $n=3$). (D) Cell lysates from experiments performed using peripheral blood mononuclear cells from two different donors were analyzed for intracellular IL-18 content by immunoblot analysis. In accord with previous data (Mehta et al., 2001), only pro-interleukin-18 was detectable in cell extract, independent of the conditions investigated.

streptomycin (Gibco-BRL, Eggenstein, Germany), and 1% (v/v) heat-inactivated human AB serum (Sigma) (Nold et al., 2003). After the indicated incubation periods, interleukin-18 was quantified in cell-free supernatants by enzyme linked immunosorbent assay (ELISA) (Diaclone, Hölzel Diagnostika, Köln, Germany). This ELISA detects pro- and mature interleukin-18. To determine intracellular levels of interleukin-18, peripheral blood mononuclear cells were lysed (300 mM NaCl, 50 mM Tris–Cl, pH 7.6, 0.5% Triton X-100, supplemented with protease inhibitor cocktail, Roche Molecular Biochemicals) and 70 μg of total protein were used to perform immunoblot analysis using a rabbit polyclonal anti-interleukin-18 antibody (Peprotech EC, London, UK). ELISA data were analyzed by paired Student's *t*-test on raw data using Sigma Plot (Jandel Scientific).

3. Results

In accord with recent publications, we observed release of interleukin-18 from peripheral blood mononuclear cells exposed to lipolysaccharide/ATP (Perregaux et al., 2000; Mehta et al., 2001) (Fig. 1A,B,C). Furthermore, we confirm that interleukin-18 is constitutively expressed in peripheral blood mononuclear cells (Fig. 1D) and that secretion induced by lipopolysaccharide/ATP is a fast process. In fact, there was no significant difference with regard to lipopolysaccharide/ATP-induced interleukin-18 levels in cell culture supernatants from experiments with incubation periods of 2 or 24 h, respectively (Fig. 1A and B). Secretion of interleukin-18 in response to lipopolysaccharide/ATP was potently suppressed by coincubation with KN-62 (Fig. 1A and B) or glyburide (Fig. 1C). The obtained dose–response curves for both agents agree with those previously reported for inhibition of lipopolysaccharide/ATP-induced interleukin-1β (Hamon et al., 1997; Grahames et al., 1999). Secretion of interleukin-18 in response to lipopolysaccharide/ATP coincided with depletion of intracellular pro-interleukin-18 as detected by immunoblot analysis. In agreement with the ELISA data on interleukin-18 secretion (Fig. 1A,B,C), we observed that coincubation with KN-62 or glyburide was able to inhibit depletion of intracellular interleukin-18 in response to lipopolysaccharide/ATP (Fig. 1D).

4. Discussion

Biochemical events that mediate release of interleukin-1β and interleukin-18 from lipopolysaccharide/ATP-activated monocytes are still poorly understood but clearly include activation of P_{2x7} receptors (Buell et al., 1998; Solle et al., 2001). Furthermore, this process appears to involve a P_{2x7}-mediated K^+efflux, an activation of caspase-1 (Perregaux and Gabel, 1994; Di Virgilio et al., 1998; Mehta et al., 2001), and the activity of ABC transporter 1 (Hamon et al., 1997). In the present study, we demonstrate for the first time suppression of lipopolysaccharide/ATP-induced interleukin-18 secretion by the P_{2x7} antagonist KN-62 and by glyburide, a potent inhibitor of ATP-sensitive K^+-channels and of the ABC transporter 1 (Hamon et al., 1997). These pharmacological data underscore the current view that maturation, processing, and release of interleukin-1β and interleukin-18 are mediated by rather similar molecular mechanisms. Besides being a potent inhibitor of P_{2x7}, KN-62 is also a recognized inhibitor of calmodulin kinase II (Williams et al., 1996). Therefore, we cannot fully exclude the possibility that this activity may contribute to effects of KN-62 on release of interleukin-1β and interleukin-18. However, data that relate ATP to interleukin-1β/interleukin-18 secretion and P_{2x7} activation are strong (Perregaux and Gabel, 1994; Mehta et al., 2001; Labasi et al., 2002) and potent inhibition of P_{2x7} function by KN-62 is a well-established observation (Humphreys et al., 1998; Grahames et al., 1999; Eschke et al., 2002). Thus, P_{2x7} antagonism imposes as prime mechanism of the inhibitory action of KN-62 described herein.

The present and previous data suggest that by targeting the process of cytokine release pharmacological agents such as KN-62 and glyburide have the potential to inhibit bioactivity of both, interleukin-1β and interleukin-18. Simultaneous inhibition of these two cytokines may be desired with regard to anti-cytokine strategies in the context of chronic inflammation and septic shock. In fact, expression of P_{2x7} is upregulated by pro-inflammatory stimuli like lipopolysaccharide/interferon-γ (Humphreys and Dubyak, 1996). The significance of ATP signaling via the P_{2x7} receptor in an inflammatory setting is furthermore highlighted by the observation that P_{2x7} knockout mice show greatly reduced lipopolysaccharide-mediated inflammatory responses in a model of experimental arthritis (Labasi et al., 2002). Taken together, P_{2x7} antagonism or modulation of ABC type of transporters by pharmacological means may represent promising strategies that target interleukin-18 secretion and thus have the potential to interfere with the pro-inflammatory cytokine cascade at a rather proximal position.

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