Biochemical and Biophysical Research Communications 463 (2015) 661–666

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Ameliorative effect of methylthiouracil on TGFBIp-induced septic responses

Byeongjin Jung [a, 1], Sae-Kwang Ku [b, 1], Jong-Sup Bae [a, *]

[a] College of Pharmacy, CMRI, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 702-701 Republic of Korea
[b] Department of Anatomy and Histology, College of Korean Medicine, Daegu Haany University, Gyeongsan 712-715, South Korea

ARTICLE INFO

Article history:
Received 15 May 2015
Accepted 30 May 2015
Available online 2 June 2015

Keywords:
Drug repositinging
Methylthiouracil
TGFBIp
Sepsis

ABSTRACT

The screening of bioactive compound libraries can be an effective approach for repositioning FDA-approved drugs or discovering new treatments for human diseases. Transforming growth factor β-induced protein (TGFBIp) is an extracellular matrix protein whose expression in several cell types is greatly increased by TGF-β. TGFBIp is released by human umbilical vein endothelial cells (HUVECs), and functions as a mediator of experimental sepsis. Here, we investigated the anti-septic effects and underlying mechanisms of methylthiouracil (MTU), used as antithyroid drug, against TGFBIp-mediated septic responses in HUVECs and mice. The anti-inflammatory activities of MTU were determined by measuring permeability, human neutrophils adhesion and migration, and activation of pro-inflammatory proteins in TGFBIp-activated HUVECs and mice. According to the results, MTU effectively inhibited lipopolysaccharide-induced release of TGFBIp, and suppressed TGFBIp-mediated septic responses, such as hyperpermeability, adhesion and migration of leukocytes, and expression of cell adhesion molecules. In addition, MTU suppressed CLP-induced sepsis lethality and pulmonary injury. Collectively, these results indicate that MTU could be a potential therapeutic agent for treatment of various severe vascular inflammatory diseases via inhibition of the TGFBIp signaling pathway.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Discoveries about the molecular basis of disease provide unprecedented opportunities to translate research findings into new medicines. However, developing a brand-new drug takes an enormous amount of time, money and effort, mainly because of bottlenecks in the therapeutic development process [1]. “Repositioning” generally refers to studying a compound or biologic (referred to as agents) to treat one disease or condition to see if it is safe and effective for treating other diseases [2,3]. Many agents approved for other uses already have been tested in humans, so detailed information is available on their pharmacology, formulation and potential toxicity. Because repositioning builds upon previous research and development efforts, new candidate therapies could be ready for clinical trials quickly, speeding their review by the FDA and, if approved, integration into health care [2,3].

Transforming growth factor β-induced protein (TGFBIp) is an extracellular matrix protein that can be highly expressed in various cell types [4–6]. TGFBIp contains an N-terminal secretory signal peptide, followed by a cysteine-rich domain, four internal homologous repeats (FAS1 domain), and a C-terminal tripeptide Arg-Gly-Asp (RGD) motif [4]. Several studies suggest TGFBIp is involved in cell growth, cell differentiation, wound healing, tumorigenesis, wound healing, and apoptosis [5–7]. Very recently, we reported that TGFBIp is a promising therapeutic target for the treatment of severe vascular inflammatory diseases, such as sepsis and septic shock [6,8]. In fact, the blockade of TGFBIp, even at later times after the onset of infection, has been shown to rescue mice from lethal sepsis [8]. TGFBIp also acts as a lethal mediator in conditions such as sepsis, in which serum TGFBIp levels are substantially increased [6,8]. Once released into the extracellular milieu, TGFBIp can bind to cell surface receptors, such as integrins αvβ3 and αvβ5 in human endothelial cells [9].

In our search for repositioning FDA-approved drugs (total 1163), 327 drugs were selected which are related to vascular inflammation

* Corresponding author. College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, 80 Dahak-ro, Buk-gu, Daegu 702-701, Republic of Korea.
E-mail address: baejs@knu.ac.kr (J.-S. Bae).
[1] First two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.05.120
0006-291X/© 2015 Elsevier Inc. All rights reserved.

and infection. Among selected drugs, high contents screening system (PerkinElmer Operetta, Waltham, MA) was used to select the compounds which modulate TGFBIp-mediated vascular endothelium disruption, and we found that methylthiouracil (MTU, used as an antithyroid drug) had anti-septic effects on TGFBIp-mediated severe inflammatory responses. Together with our previous reports, which demonstrated the potential effects of TGFBIp on vascular inflammatory responses [6,8], this study was conducted in an effort to understand the mechanism of the anti-septic action of MTU by investigating their anti-septic effect on TGFBIp- or CLP-induced septic responses in human endothelial cells and mice.

2. Materials and methods

2.1. Reagents

Methylthiouracil (MTU) was purchased from Abcam (Cambridge, MA). Evans blue, and crystal violet were obtained from Sigma (St. Louis, MO). Vybrant DiD (used at 5 μM) was obtained from Invitrogen (Carlsbad, CA). TGFBIp protein was purified as described previously [9].

2.2. Cell culture

Primary HUVECs were obtained from Cambrex Bio Science (Charles City, IA) and maintained as described previously [9–11]. Briefly, the cells were cultured to confluency at 37 °C and 5% CO_2 in EBM-2 basal media supplemented with growth supplements (Cambrex Bio Science). All experiments were carried out with HUVEC at passage 3–5. Human neutrophils were freshly isolated from whole blood (15 mL) obtained by venipuncture from five healthy volunteers, and maintained as previously described [12].

2.3. ELISA for TGFBIp

TGFBIp concentrations in cell culture media or mouse serum were determined by competitive ELISA, as described previously [6,9].

2.4. In vivo permeability and leukocyte migration assay

For *in vivo* study, male mice were anesthetized with 2% isoflurane (Forane, JW pharmaceutical, South Korea) in oxygen delivered via a small rodent gas anesthesia machine (RC2, Vetequip, Pleasanton, CA), first in a breathing chamber and then via a facemask. They were allowed to breath spontaneously during the procedure. Mice were treated with TGFBIp (0.1 mg/kg, i.v.) for 6 h followed by treatment with MTU (142 or 284 μg/kg) for 6 h.

For *in vivo* permeability assay, 1% Evans blue dye solution in normal saline was injected intravenously into each mouse. Thirty minutes later, mice were euthanized and peritoneal exudates were collected by washing cavities with 5 mL of normal saline and centrifuging at 200 g for 10 min. Absorbance of supernatants was read at 650 nm. Vascular permeabilities are expressed as μg of dye/mouse that leaked into the peritoneal cavity, and were determined using a standard curve, as previously described [13,14].

For assessment of leukocyte migration, mice were euthanized after 6 h and peritoneal cavities were washed with 5 mL of normal saline. Samples (20 μl) of peritoneal fluids obtained were mixed with 0.38 mL of Turk's solution (0.01% crystal violet in 3% acetic acid) and numbers of leukocytes were counted under a light microscope.

2.5. Statistical analysis

All experiments were performed independently at least three times. Values are expressed as mean ± standard error of the mean (SEM). The statistical significance of differences between test groups was evaluated by one-way analysis of variance (ANOVA) and Tukey's post-hoc test. Kaplan–Meier survival analysis was performed for evaluation of overall survival rates. SPSS for Windows, version 16.0 (SPSS, Chicago, IL) was used to perform statistical analysis, and statistical significance was accepted for p values < 0.05.

* The online supplement provides more information regarding methods.

3. Results and discussion

Recently the issue of repositioning FDA-approved drugs for new indications has gained significant attention as a result of the time and cost necessary in bringing a novel drug into clinical use [2,3,15]. Here we report the results of MTU, as antithyroid drug, on release of TGFBIp and TGFBIp-mediated vascular barrier disruptive response were determined *in vitro* and *in vivo*.

3.1. Effects of MTU on LPS and CLP-mediated release of TGFBIp

Our previous study have demonstrated stimulation of TGFBIp release by LPS from human endothelial cells, and 100 ng/mL LPS is sufficient to induce release of TGFBIp [8,9]. Similarly, in the current study, 100 ng/mL LPS stimulated release of TGFBIp by HUVECs (Fig. 1A). To investigate the effects of MTU on LPS-mediated release of TGFBIp, HUVECs were stimulated with 100 ng/mL LPS for 1 h, followed by treated with increasing concentrations of MTU for 6 h. As shown in Fig. 1A, MTU inhibited release of TGFBIp in HUVECs, with an optimal effective concentration >5 μM. However, in the absence of LPS pretreatment, MTU did not affect TGFBIp release (Fig. 1A). In order to confirm these effects *in vivo*, CLP-induced septic mice were used, because this model more closely resembles human sepsis than LPS-induced endotoxemia [16]. As shown in Fig. 1B, treatment with MTU resulted in marked inhibition of CLP-induced release of TGFBIp. The average circulating blood volume for mice is 72 mL/kg [17]. Because the average weight of used mouse is 27 g, and the average blood volume is 2 mL, the amount of MUT (142 or 284 μg/kg) injected yielded a maximum concentration of 10 or 20 μM in the peripheral blood. To determine the molecular mechanism by which MTU inhibited the release of LPS-mediated TGFBIp, we tested the effects of MTU on the transcriptional regulation of TGFBIp by LPS in HUVECs. Thus, we measured the effect of MTU on LPS-induced TGFBIp mRNA levels using real time qRT-PCR. As shown in Fig. 1C, LPS induced an increase in the expression levels of TGFBIp mRNA and treatment with MTU resulted in decreased expression levels of LPS-induced TGFBIp mRNA.

Next, we investigated the effects of MTU on expression of the TGFBIp receptors, integrin αvβ3 and αvβ5 in HUVECs [9]. As shown in Fig. 1C, treatment with LPS resulted in over four fold increase in expression of αvβ5 in HUVECs, and treatment with MTU resulted in significantly inhibited expression of αvβ5. However, consistent to previous report [9], the expression of integrin αvβ3 was not changed by LPS [9] nor MTU (Fig. 1D). Therefore, the inhibitory effects of MTU on release of TGFBIp were mediated by suppression of TGFBIp receptor (integrin αvβ5).

To assess the cytotoxicity of MTU, cell viability assays were performed in HUVECs treated with MTU for 24 h. At concentrations up to 50 μM, MTU did not affect cell viability (data not shown). High plasma concentrations of TGFBIp in patients with sepsis are known

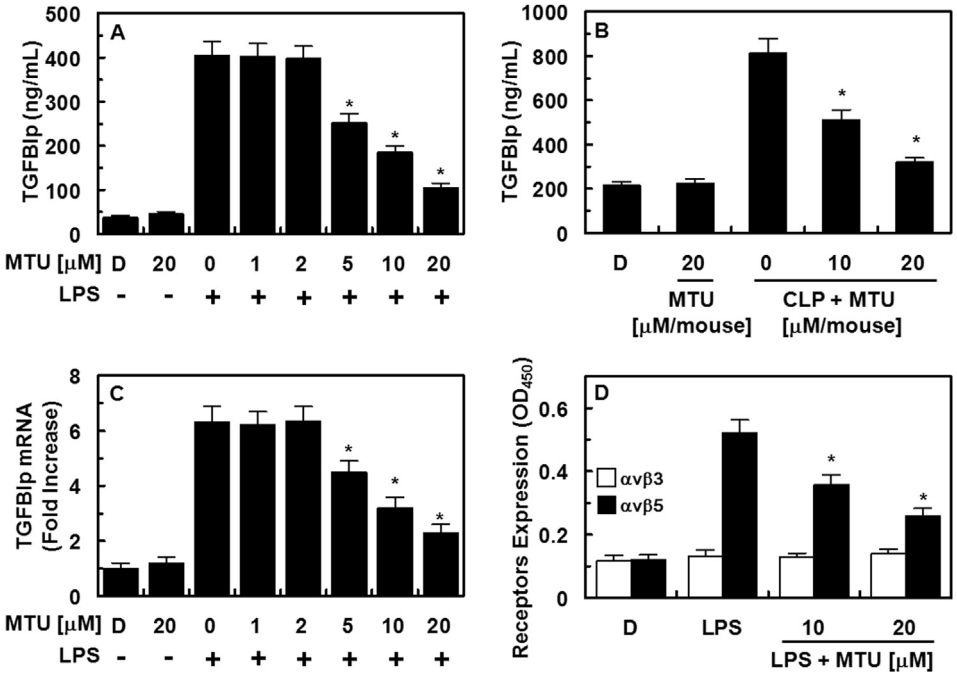

Fig. 1. Effects of MTU on release of TGFBIp and expression of receptors. (A) HUVECs were treated with the indicated concentrations of MTU for 6 h, after stimulated with LPS (100 ng/mL, 1 h), and TGFBIp release was measured by ELISA. (B) Male C57BL/6 mice that underwent CLP were administered MTU at 10 or 20 μM/mouse each intravenously 12 h after CLP (black bar, n = 5). Mice were euthanized 24 h after CLP. Serum TGFBIp levels were measured by ELISA. (C) The same as (A) except that real time qRT-PCR analysis was performed using specific primers for TGFBIp and actin, as described in the materials and methods section. (D) Confluent HUVECs were activated with LPS (100 ng/mL, 3 h), followed by incubation with MTU for 6 h. Expression of αvβ3 (white bar) and αvβ5 (black bar) was determined by cell-based ELISA. D = 0.2% DMSO is the vehicle control. *$p < 0.05$ *versus* LPS alone (A, C, D) or CLP alone (B).

to be related to the severity of sepsis [9] and pharmacological inhibition of TGFBIp is known to improve survival in animal models of sepsis [8]. Therefore, prevention of CLP-induced release of TGFBIp by MTU suggests the potential for use of MTU in treatment of vascular inflammatory diseases.

3.2. Effect of MTU on TGFBIp-mediated vascular barrier disruption

A permeability assay was performed to determine the effects of MTU on the barrier integrity of HUVECs. Treatment with 20 μM MTU alone did not alter barrier integrity (Fig. 2A). In contrast, TGFBIp is known to cause cleavage and disruption of endothelial barrier integrity [8,9]. Thus, HUVECs were treated with various concentrations of MTU for 6 h after addition of TGFBIp (5 μg/mL). As shown in Fig. 2A, treatment with MTU resulted in a dose-dependent decrease in TGFBIp-mediated disruption of barrier integrity. TGFBIp-mediated vascular permeability in mice was assessed in order to confirm this vascular barrier protective effect *in vivo*. As shown in Fig. 2B, treatment with MTU resulted in markedly inhibited peritoneal leakage of dye induced by TGFBIp.

Cytoskeletal proteins are important for the maintenance of cell integrity and shape [18]. In addition, redistribution of the actin cytoskeleton, detachment of cells, and loss of cell–cell contact due to cytokine stimulation are all associated with an increased endothelial monolayer permeability [19,20]. Therefore, we next examined the effects of MTU on actin cytoskeletal arrangement in HUVECs by immunofluorescence staining of HUVEC monolayers with F-actin-labeled fluorescein phalloidin. Control HUVECs exhibited a random distribution of F-actin throughout the cells, with some localization of actin filament bundles at the cell boundaries (Fig. 2C). Barrier disruption in HUVECs induced by TGFBIp treatment (5 μg/mL) was accompanied by the formation of paracellular gaps (shown by arrows). In addition, treatment with MTU (10 or 20 μM) inhibited the formation of TGFBIp-induced paracellular gaps with the formation of dense F-actin rings (Fig. 2C). These results suggest that MTU treatment inhibited the TGFBIp-mediated morphological changes and gap formation in endothelial cells, which are associated with F-actin redistribution, thereby increasing vascular barrier integrity.

Sepsis induces, such as high mobility group box 1 (HMGB1) and LPS, are known to induce pro-inflammatory responses by promoting phosphorylation of p38 MAPK [21–24]. Therefore, we determined whether TGFBIp could also enhance the phosphorylation of p38 MAPK, if so, determined whether MTU inhibit TGFBIp-induced activation of p38 MAPK in HUVECs. As shown in Fig. 2D, TGFBIp induced the activation of p38 MAPK, which was significantly inhibited by treatment with MTU. These findings demonstrate inhibition of TGFBIp-mediated endothelial disruption and maintenance of human endothelial cell barrier integrity by MTU in mice treated with TGFBIp.

3.3. Effects of MTU on TGFBIp-mediated CAMs expression, neutrophils adhesion, and migration

Several studies have report that TGFBIp enhanced the expression of CAMs, such as ICAM-1, VCAM-1, and E-selectin, on the surfaces of human cells, thereby promoting adhesion and migration of leukocytes across the endothelium to sites of inflammation [6,9,25,26]. According to our findings, TGFBIp induced up-regulation of the surface expression of VCAM-1, ICAM-1, and E-Selectin (Fig. 3A) and MTU inhibited this effect, suggesting that the inhibitory effects of MTU on expression of CAMs are mediated via attenuation of the TGFBIp signaling pathway by MTU. In addition, elevated expression of CAMs corresponded well with enhanced binding of human neutrophils to TGFBIp-activated endothelial cells, followed by their migration. In addition, treatment with MTU

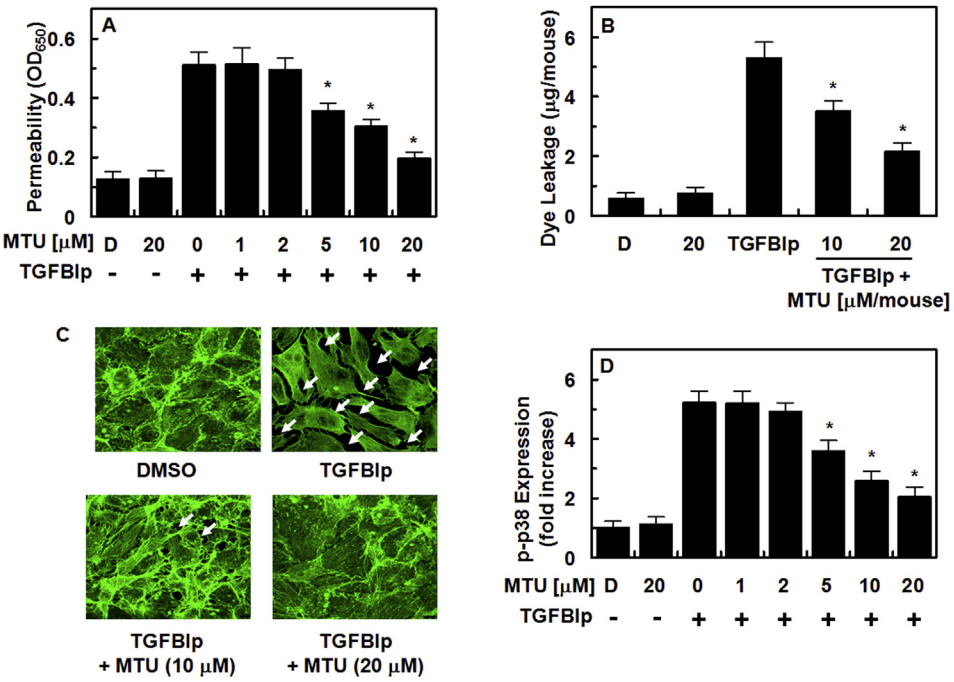

Fig. 2. Effects of MTU on TGFBIp-mediated permeability *in vitro* and *in vivo*. The effects of post-treatment with different concentrations of MTU for 6 h on the barrier disruptions caused by TGFBIp (A, 5 μg/mL, 6 h) were monitored by measuring the flux of Evans blue bound albumin across HUVECs. (B) The effects of MTU at 10 or 20 μM/mouse on TGFBIp-induced (0.1 mg/kg, i.v.) vascular permeability in mice were examined by measuring the amount of Evans blue in peritoneal washings (expressed μg/mouse, n = 5). (C) Staining for F-actin. HUVEC monolayers grown on glass coverslips were stimulated with TGFBIp for 1 h, and then treated with MTU for 6 h, and stained for F-actin. Arrows indicate intercellular gaps. (D) HUVECs were activated with TGFBIp (5 μg/mL, 6 h), followed by treated with different concentrations of MTU for 6 h. The effects of MTU on TGFBIp-mediated expression of phospho p38 were determined by ELISA. Results are expressed as the mean ± SEM of at least three independent experiments. D = 0.2% DMSO is the vehicle control. *$p < 0.05$ *versus* TGFBIp alone.

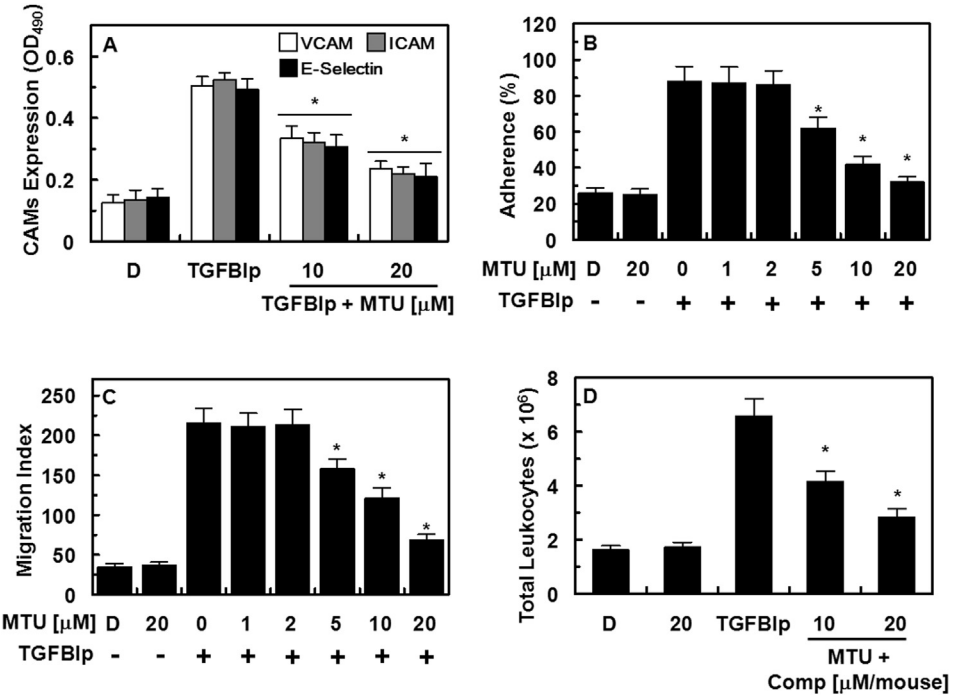

Fig. 3. Effects of MTU on TGFBIp-mediated pro-inflammatory responses. (A–C), HUVECs were stimulated with TGFBIp (5 μg/mL) for 6 h, followed by treatment with MTU for 6 h. TGFBIp-mediated (A) expression of VCAM-1 (white bar), ICAM-1 (gray bar), and E-selectin (black bar) in HUVECs, (B) adherence of human neutrophils to HUVEC monolayers, and (C) migration of human neutrophils through HUVEC monolayers were analyzed. (D) The effects of post-treatment with MTU at 10 or 20 μM/mouse on leukocyte migration into the peritoneal cavities of mice caused by TGFBIp (0.1 mg/kg, i.v.) were analyzed. All results indicate the mean ± SEM of three separate experiments (n = 5). D = 0.2% DMSO is the vehicle control. *$p < 0.05$ *vs*. TGFBIp.

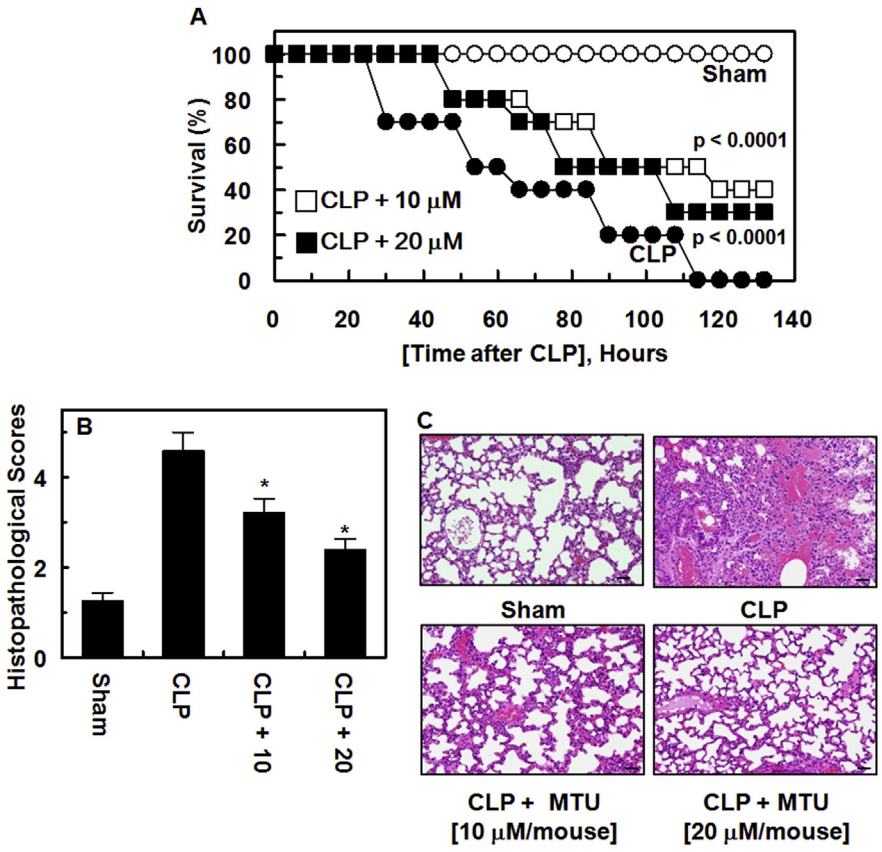

Fig. 4. Effects of MTU on lethality or pulmonary injury after CLP. (A) Male C57BL/6 mice (n = 20) were administered MTU (10 μM/mouse, □; 20 μM/mouse, ■) at 12 h and 50 h after CLP. Animal survival was monitored every 6 h after CLP for 132 h. Control CLP mice (●) and sham-operated mice (○) were administered sterile saline (n = 20). Kaplan–Meier survival analysis was used for determination of overall survival rates *versus* CLP treated mice. (B) The same as (A) except that mice were euthanized 96 h after CLP. Histopathological scores of the lung tissue were recorded as described in methods. *indicates $p < 0.05$ vs. CLP. (C) Photomicrographs of lung tissues (H&E staining, X200). Sham group (grade 1); CLP group (grade 3); Right, CLP + MTU (10 or 20 μM/mouse) (grade 2). Illustrations indicate representative images from three independent experiments.

resulted in down-regulation of human neutrophils adherence and their subsequent migration across activated endothelial cells in a concentration-dependent manner (Fig. 3B and C). These results suggest that MTU not only inhibit endotoxin-mediated release of TGFBIp in endothelial cells, but also down-regulate the pro-inflammatory signaling effect caused by release of TGFBIp, thereby inhibiting amplification of inflammatory pathways by nuclear cytokines. To confirm this effect *in vivo*, we examined TGFBIp-induced migration of leukocytes in mice. TGFBIp was found to stimulate migration of leukocytes into the peritoneal cavities of mice, and treatment with MTU resulted in a significant reduction of peritoneal leukocyte counts (Fig. 3D). Experiments on CAMs are widely performed *in vitro* for study of regulation of the interactions between leukocytes and endothelial cells [27,28]. In the current study, treatment with MTU resulted in down-regulation of TGFBIp-induced levels of VCAM-1, ICAM-1, and E-selectin, suggesting that MTU inhibit the adhesion and migration of leukocytes to inflamed endothelium.

3.4. Protective effect of MTU in CLP-induced septic mortality

Sepsis is a systemic response to serious infection, and has a poor prognosis when it is associated with organ dysfunction, hypoperfusion, or hypotension [29,30]. Based on the above-described findings, we hypothesized that treatment with MTU would result in reduced mortality in our CLP-induced sepsis mouse model. To investigate the question of whether MTU protects mice from CLP-induced sepsis lethality, MTU was administered to mice after CLP. Twenty four hours after the operation, animals manifested signs of sepsis, such as shivering, bristled hair, and weakness. Administration of MTU (142 or 284 μg/kg) 12 h after CLP did not prevent CLP-induced death (data not shown); therefore, they were administered two times (once 12 h after CLP and once 50 h after CLP), which resulted in an increase in the survival rate from 30 to 40%, according to Kaplan–Meier survival analysis ($p < 0.0001$, Fig. 4A). This marked survival benefit achieved by administration of MTU suggests that suppression of TGFBIp release and of TGFBIp-mediated inflammatory responses provides a therapeutic strategy for management of sepsis and septic shock.

3.5. Protective effect of MTU in the CLP-induced pulmonary injury

To confirm the protective effects of MTU on CLP-induced death, we determined the effects of MTU on CLP-induced pulmonary injury. There were no significant differences between lungs of sham and sham + MTU in light microscopic observations (data not shown). In the CLP group, interstitial edema with massive infiltration of the inflammatory cells into the interstitium and alveolar spaces were observed and the pulmonary architecture was severely damaged (Fig. 4B and C). These morphological changes were less pronounced in the CLP + MTU group (Fig. 4B and C).

The most frequent adverse effect of MTU is granulocytopenia [31]. Despite the known side effect of MTU, in this study, we found that MTU did not affect the number of granulocytes and macrophages in the blood in sham mice treated with MTU (data not shown). Although we could not rule out any possibilities of the

difference species, the discrepancy may be explained by the administrated dosage of MTU; MTU was used for antithyroid at 200 mg/day in human (average body weight; 70 kg), however, in this study, MTU was used at 142 μg/kg (9.94 mg/70 kg) in mice.

The molecular mechanism of anti-inflammatory effects of MTU against TGFBIp-mediated septic response may be mediated by the suppression of TGFBIp release and transcriptional suppression of TGFBIp mRNA (Fig. 1A–C), the expressions of TGFBIp receptor (integrin αvβ5, Fig. 1D) and TGFBIp-mediated hyperpermeability (Fig. 2A–C) via suppression of the activation of p38 (Fig. 2D). Furthermore, the inhibitory mechanism of MTU on the interaction of between leukocytes and endothelial cells is mediated by the inhibition of the expressions of CAMs such as VCAM, ICAM, and E-Selectin (Fig. 3). The possible main target of MTU on TGFBIp-mediated septic response would be the interactions between released TGFBIp and its receptor (integrin αvβ5) because the binding of ligand (TGFBIp) to its receptor (αvβ5) mediated downstream severe vascular inflammatory responses such as hyperpermeabililty, adhesion and migration of leukocytes toward endothelial cells [8,9]. Based on current findings, MTU merit use as potential therapeutic agents for severe vascular inflammatory diseases, such as sepsis and septic shock, beyond.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2012R1A5A2A42671316) and by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI15C0001).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.05.120.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.05.120.

References

[1] M. Dickson, J.P. Gagnon, The cost of new drug discovery and development, Discov. Med. 4 (2004) 172–179.
[2] B.M. Padhy, Y.K. Gupta, Drug repositioning: re-investigating existing drugs for new therapeutic indications, J. Postgrad. Med. 57 (2011) 153–160.
[3] K.A. O'Connor, B.L. Roth, Finding new tricks for old drugs: an efficient route for public-sector drug discovery, Nat. Rev. Drug Discov. 4 (2005) 1005–1014.
[4] J. Skonier, M. Neubauer, L. Madisen, K. Bennett, G.D. Plowman, A.F. Purchio, cDNA cloning and sequence analysis of beta ig-h3, a novel gene induced in a human adenocarcinoma cell line after treatment with transforming growth factor-beta, DNA Cell. Biol. 11 (1992) 511–522.
[5] J. Skonier, K. Bennett, V. Rothwell, S. Kosowski, G. Plowman, P. Wallace, S. Edelhoff, C. Disteche, M. Neubauer, H. Marquardt, et al., beta ig-h3: a transforming growth factor-beta-responsive gene encoding a secreted protein that inhibits cell attachment in vitro and suppresses the growth of CHO cells in nude mice, DNA Cell. Biol. 13 (1994) 571–584.
[6] J.S. Bae, S.H. Lee, J.E. Kim, J.Y. Choi, R.W. Park, J. Yong Park, H.S. Park, Y.S. Sohn, D.S. Lee, E. Bae Lee, I.S. Kim, Betaig-h3 supports keratinocyte adhesion, migration, and proliferation through alpha3beta1 integrin, Biochem. Biophys. Res. Commun. 294 (2002) 940–948.
[7] J.E. Kim, S.J. Kim, H.W. Jeong, B.H. Lee, J.Y. Choi, R.W. Park, J.Y. Park, I.S. Kim, RGD peptides released from beta ig-h3, a TGF-beta-induced cell-adhesive molecule, mediate apoptosis, Oncogene 22 (2003) 2045–2053.
[8] J.S. Bae, W. Lee, H.N. Son, Y.M. Lee, I.S. Kim, Anti-transforming growth factor beta-induced protein antibody ameliorates vascular barrier dysfunction and improves survival in sepsis, Acta Physiol. (Oxf) 212 (2014) 306–315.
[9] J.S. Bae, W. Lee, J.O. Nam, J.E. Kim, S.W. Kim, I.S. Kim, Transforming growth factor beta-induced protein promotes severe vascular inflammatory responses, Am. J. Respir. Crit. Care Med. 189 (2014) 779–786.
[10] S.K. Ku, M.S. Han, M.Y. Lee, Y.M. Lee, J.S. Bae, Inhibitory effects of oroxylin A on endothelial protein C receptor shedding in vitro and in vivo, BMB Rep. 47 (2014) 336–341.
[11] S.K. Ku, J.S. Bae, Antithrombotic activities of sulforaphane via inhibiting platelet aggregation and FIIa/FXa, Arch. Pharm. Res. 37 (2014) 1454–1463.
[12] R. Hofbauer, D. Moser, H. Salfinger, M. Frass, S. Kapiotis, Sufentanil inhibits migration of human leukocytes through human endothelial cell monolayers, Anesth. Analg. 87 (1998) 1181–1185.
[13] J.S. Bae, W. Lee, A.R. Rezaie, Polyphosphate elicits proinflammatory responses that are counteracted by activated protein C in both cellular and animal models, J. Thromb. Haemost. 10 (2012) 1145–1151.
[14] J.D. Lee, J.E. Huh, G. Jeon, H.R. Yang, H.S. Woo, D.Y. Choi, D.S. Park, Flavonol-rich RVHxR from Rhus verniciflua stokes and its major compound fisetin inhibits inflammation-related cytokines and angiogenic factor in rheumatoid arthritic fibroblast-like synovial cells and in vivo models, Int. Immunopharmacol. 9 (2009) 268–276.
[15] X. Liu, F. Zhu, X.H. Ma, Z. Shi, S.Y. Yang, Y.Q. Wei, Y.Z. Chen, Predicting targeted polypharmacology for drug repositioning and multi- target drug discovery, Curr. Med. Chem. 20 (2013) 1646–1661.
[16] J.A. Buras, B. Holzmann, M. Sitkovsky, Animal models of sepsis: setting the stage, Nat. Rev. Drug Discov. 4 (2005) 854–865.
[17] K.H. Diehl, R. Hull, D. Morton, R. Pfister, Y. Rabemampianina, D. Smith, J.M. Vidal, C. van de Vorstenbosch, A good practice guide to the administration of substances and removal of blood, including routes and volumes, J. Appl. Toxicol. 21 (2001) 15–23.
[18] H.J. Schnittler, S.W. Schneider, H. Raifer, F. Luo, P. Dieterich, I. Just, K. Aktories, Role of actin filaments in endothelial cell-cell adhesion and membrane stability under fluid shear stress, Pflugers Arch. 442 (2001) 675–687.
[19] J. Friedl, M. Puhlmann, D.L. Bartlett, S.K. Libutti, E.N. Turner, M.F. Gnant, H.R. Alexander, Induction of permeability across endothelial cell monolayers by tumor necrosis factor (TNF) occurs via a tissue factor-dependent mechanism: relationship between the procoagulant and permeability effects of TNF, Blood 100 (2002) 1334–1339.
[20] I. Petrache, A. Birukova, S.I. Ramirez, J.G. Garcia, A.D. Verin, The role of the microtubules in tumor necrosis factor-alpha-induced endothelial cell permeability, Am. J. Respir. Cell. Mol. Biol. 28 (2003) 574–581.
[21] Y.H. Qin, S.M. Dai, G.S. Tang, J. Zhang, D. Ren, Z.W. Wang, Q. Shen, HMGB1 enhances the proinflammatory activity of lipopolysaccharide by promoting the phosphorylation of MAPK p38 through receptor for advanced glycation end products, J. Immunol. 183 (2009) 6244–6250.
[22] C. Sun, C. Liang, Y. Ren, Y. Zhen, Z. He, H. Wang, H. Tan, X. Pan, Z. Wu, Advanced glycation end products depress function of endothelial progenitor cells via p38 and ERK 1/2 mitogen-activated protein kinase pathways, Basic Res. Cardiol. 104 (2009) 42–49.
[23] P.R. Mittelstadt, J.M. Salvador, A.J. Fornace Jr., J.D. Ashwell, Activating p38 MAPK: new tricks for an old kinase, Cell. Cycle 4 (2005) 1189–1192.
[24] K.L. Walton, L. Holt, R.B. Sartor, Lipopolysaccharide activates innate immune responses in murine intestinal myofibroblasts through multiple signaling pathways, Am. J. Physiol. Gastrointest. Liver Physiol. 296 (2009) G601–G611.
[25] S.H. Lee, J.S. Bae, S.H. Park, B.H. Lee, R.W. Park, J.Y. Choi, J.Y. Park, S.W. Ha, Y.L. Kim, T.H. Kwon, I.S. Kim, Expression of TGF-beta-induced matrix protein betaig-h3 is up-regulated in the diabetic rat kidney and human proximal tubular epithelial cells treated with high glucose, Kidney Int. 64 (2003) 1012–1021.
[26] S.W. Park, J.S. Bae, K.S. Kim, S.H. Park, B.H. Lee, J.Y. Choi, J.Y. Park, S.W. Ha, Y.L. Kim, T.H. Kwon, I.S. Kim, R.W. Park, Beta ig-h3 promotes renal proximal tubular epithelial cell adhesion, migration and proliferation through the interaction with alpha3beta1 integrin, Exp. Mol. Med. 36 (2004) 211–219.
[27] W.N. Lin, S.F. Luo, C.B. Wu, C.C. Lin, C.M. Yang, Lipopolysaccharide induces VCAM-1 expression and neutrophil adhesion to human tracheal smooth muscle cells: involvement of Src/EGFR/PI3-K/Akt pathway, Toxicol. Appl. Pharmacol. 228 (2008) 256–268.
[28] M.P. Ruiz-Torres, G. Perez-Rivero, M. Rodriguez-Puyol, D. Rodriguez-Puyol, M.L. Diez-Marques, The leukocyte-endothelial cell interactions are modulated by extracellular matrix proteins, Cell. Physiol. Biochem. 17 (2006) 221–232.
[29] J.S. Bae, Role of high mobility group box 1 in inflammatory disease: focus on sepsis, Arch. Pharm. Res. 35 (2012) 1511–1523.
[30] J. Cohen, The immunopathogenesis of sepsis, Nature 420 (2002) 885–891.
[31] J.H. Scharf, R. Schmidt, D. Marzotko, E. Mantel, C. Wanka, Side effects of single and combined administration of methylthiouracil, p-hydroxypropiophenone, diiodothyrosine and alloxan on the blood cell count, hemoglobin content and blood sugar level in albino rats, Endokrinologie 47 (1965) 161–171.