Mini Review

Effect of glucosamine on Interleukin-8 production from human colonic epithelial cell line

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Intestinal epithelial cell (IEC) is the first line of mucosal surface that faces the challenge of exogenous pathogens. IEC play a pivotal role in host defense by sensing microbial infection, resulting in the production of proinflammatory cytokines that affect leukocyte functions. Glucosamine, a naturally occurring amino monosaccharide, has been shown to inhibit neutrophil activation. However, it has not been examined whether glucosamine affects the IEC functions. In this study to investigate the effect of glucosamine on IEC, we used a human colon cancer cell line (HT-29) and stimulated with LPS and IL-1β to induce IL-8 production. Glucosamine significantly inhibited the LPS- and IL-1β-induced IL-8 production from HT-29. Further, the effect of glucosamine on LPS- and IL-1β-binding to the cells was examined by using biotinyled-LPS and ^{125}I-labeled IL-1β; however, glucosamine did not essentially affect the LPS- and IL-1β-binding to HT-29. Furthermore, we investigated the effect of glucosamine on the phosphorylation of p38 MAPK. Glucosamine inhibited IL-1β-induced phosphorylation of p38 MAPK. These observations indicate that glucosamine inhibits IL-8 production from HT-29 cells by affecting the p38 MAPK-mediating signaling pathway, downstream of the ligand binding to the receptors.

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Introduction

Intestinal epithelial cells (IECs) play an important role in the mucosal immune system during gut inflammation, and receive their activating signals from basically two sources; 1) the humoral factors, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), produced in the activated immune cells of the gut, and 2) bacteria and bacterial products[1,2]. IL-1β and TNF-α are potent activators of IECs to produce interleukin-8 (IL-8). IL-8 is an important mediator of inflammation that belongs to the CXC chemokine family and recruits neutrophils into the inflamed tissue[3,4]. Proinflammatory cytokines, such as IL-8, IL-1 and TNF-α plays a central role in the initiation and maintenance of inflammatory responses in the inflammatory bowel disease[5-8]. IL-8 expression was up-regulated by IL-1β and TNF-α stimulation in human epithelial cells via mitogen-activated protein kinase (MAPK) phosphorylation and nuclear factor-kappa

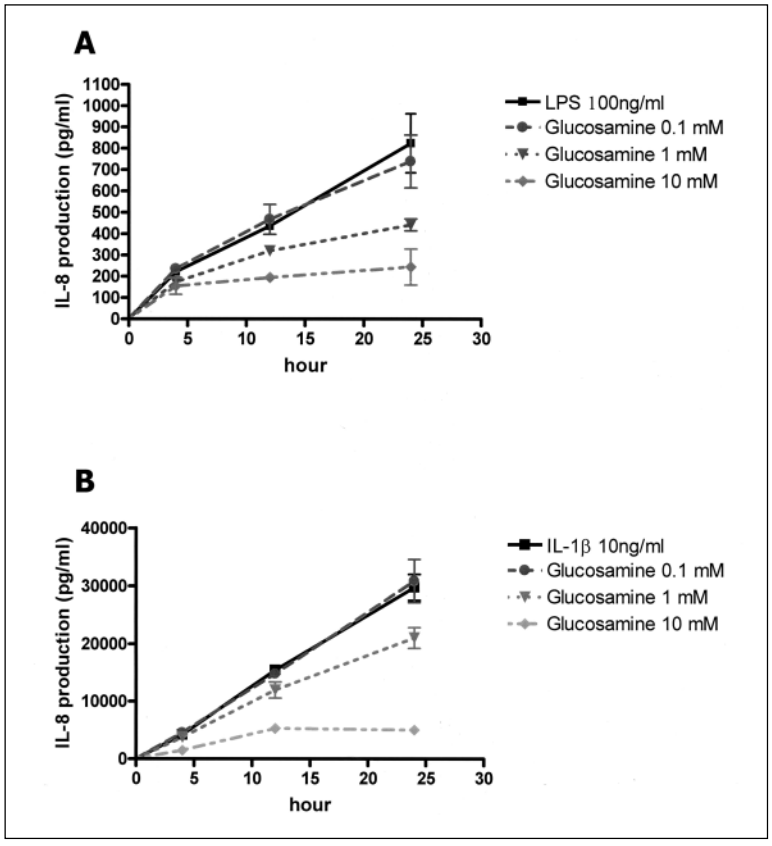

Fig.1 Effect of glucosamine on IL-8 production from HT-29 cells

HT-29 cells were incubated with 200 μl of medium containing (A) lipopolysaccharide (LPS, 100 ng/ml); (B) IL-1β (10 ng/ml) for 4, 12 and 24h in the absence or presence of 0.1 ～ 10 mM glucosamine. IL-8 protein level was determined in culture supernatants. Values are means ± SD.

B (NF-κB) activation. Furthermore, MAPKs and NF-κB were significantly activated in inflammatory bowel disease (IBD)[9]. Since the etiology of IBD, including ulcerative colitis (UC) is unknown, current treatment of this disease is based on the non-specific suppression of immune reaction[10].

Glucosamine, a naturally occurring amino monosaccharide, is present in the connective and cartilage tissues, and contributes to maintaining the strength, flexibility and elasticity of these tissues. Thus, glucosamine has been widely used to treat osteoarthritis in human[11]. Several clinical trials in osteoarthritis have shown the significant symptom-modifying effect of glucosamine[12]. According to the recent biochemical and pharmacological studies, administration of glucosamine normalizes cartilage metabolism, so as to stimulate the synthesis and inhibits the degradation of proteoglycans, and to restore the articular function[13,14].

In addition to its chondroprotective action, glucosamine is expected to exert anti-inflammatory actions by inhibiting inflammatory cell functions. In this context, Hua et al. have revealed that glucosamine suppresses neutrophil functions such as superoxide generation, phagocytosis, granule enzyme release and chemotaxis[15]. Moreover, glucosamine is demonstrated to suppress the activation of T-lymphoblasts and dendritic cells *in vitro*, and prolong the allogeneic cardicac allograft survival *in vivo*[16]. In addition, glucosamine effectively inhibits interferon-γ, IL-17 and NO production from splenocytes in a model with experimental autoimmune encephalomyelitis mice[17]. Furthermore, glucosamine has been reported to suppress the ADP-mediated platelet activation by influencing the P2Y12-mediated response[18].

In the present study, we investigated whether glucosamine suppresses IL-1β- or LPS-induced cytokine production from HT-29 human colonic epithelial cells.

Effect of glucosamine treatment on IL-8 production from HT-29

We first examined the effect of glucosamine on the cytokine production from HT-29 cells. The cytokine production from HT-29 human colon epithelial cells was evaluated as described previously[19]. In brief, HT-29 cells were cultured in McCoy's 5A medium supplemented with 10% heat-inactivated FBS (endotoxin level < 0.03 ng/ml), penicillin (100 IU/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM). After stimulation with LPS (100 ng/ml) or IL-1β (10 ng/ml, endotoxin level <1 pg/ml), the culture supernatants from HT-29 were harvested and the levels of IL-8, IL-6 and TNF-α were measured by en-

Table 1 Effect of glucosamine on the expression of CD14 in HT-29 cells

	Fluorescence intensity
Control	52.1
+ 0.1 mM Glucosamine	55.0
+ 1 mM Glucosamine	53.7
+ 10 mM Glucosamine	56.8

HT-29 cells (1×10^6 cells/ml) were incubated for 30 min at 37℃ in McCoy's 5A medium-1% FBS in the absence (control) or presence of glucosamine, and then immunostained using anti-CD14 (MY4) mAb.

Table 2 Effect of glucosamine on the binding of LPS to HT-29 cells

	Fluorescence intensity
Control	16.7
+ 0.1 mM Glucosamine	14.6
+ 1 mM Glucosamine	16.5
+ 10 mM Glucosamine	18.7

HT-29 cells (1×10^6 cells/ml) were incubated for 30 min at 37 in McCoy's 5A medium-1% FBS in the absence (control) or presence of glucosamine, and then added 100ng/ml of biotinyled-LPS. The binding of LPS to HT-29 cells were measured by Flowcytometry.

zyme linked immunosorbent assays (ELISA). IL-8 production was detected after stimulation with LPS or IL-1β for 4h, 12h, 24h and the level of IL-8 was gradually increased in a time-dependent manner (Fig.1). In contrast, neither IL-6 nor TNF-α were detected in the culture supernatants of LPS- or IL-1β-stimulated HT-29 cells (data not shown). These observations indicate that the production of IL-6 and TNF-α is much lower than that of IL-8 in HT-29 cells, as previously suggested[20]. Next, we investigated the effect of glucosamine on the LPS- or IL-1β-induced IL-8 production from HT-29 cells. HT-29 cells were exposed for 30 min with glucosmaine at the concentrations of 0.1, 1 or 10 mM before stimulation with LPS or IL-1β. Glucosamine (1,10 mM) dose-dependently inhibited the LPS- or IL-1-β-induced IL-8 production. Of note, pretreatment with 10 mM glucosamine remarkably inhibited the IL-8 production from HT-29 cells (Fig.1).

Effect of glucosamine on expression of CD14 and binding of LPS

The activation mechanism of cells by LPS includes the binding of LPS to LPS binding protein (LBP), which accelerates the transfer of LPS to CD14, the primary receptor of LPS. The LPS-CD14 complex initiates intracellular signaling by interacting with the transmembrane protein Toll like receptor-4, resulting in the production and secretion of proinflammatory cytokines[21]. Next, we examined the effect of glucosamine on the expression of CD14 by flow cytometry. HT-29 cells were treated with various concentrations (0.1～10 mM) of glucosamine for 24h. HT-29 cells expressed CD14; however, the concentrations of glucosamine examined had no effect on the expression of CD14 (Table 1). In addition, we investigated whether glucosamine affects the binding of LPS with HT-29 cells by flow cytometry. Treatment of HT-29 cells with various concentrations (0.1 ～ 10 mM) of glucosamine for 30 min did not affect the binding of LPS to HT-29 cells (Table 2). Moreover, incubation of HT-29 cells with glu-

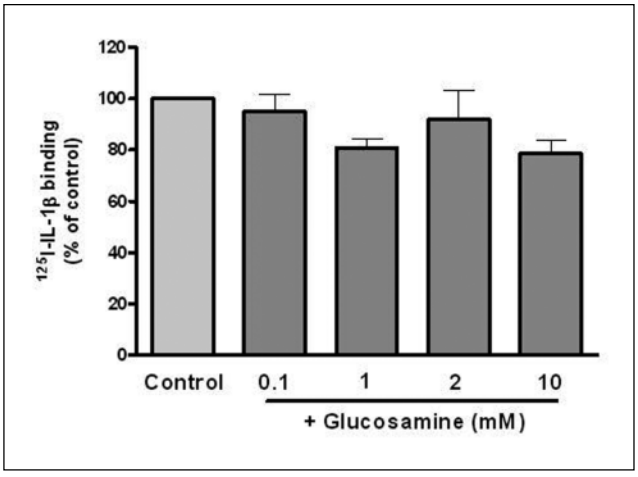

Fig.2 Effect of glucosamine on the binding of ^{125}I-IL-1β to HT-29 cells

HT-29 cells were cultured to confluency and incubated with 20 pM of ^{125}I-IL-1β in the absence or presence of 0.1 ～ 10 mM glucosamine for 4h at 4℃. HT-29 cells were washed three times with PBS, trypsinized, and then transferred to tubes and counted by γ-counter. The binding of ^{125}I-IL-1β was expressed as a percentage of that obtained with HT-29 cells (control) incubated in the absence of glucosamine. Data represent the mean ± SD.

cosamine for 24h had no effect the binding of LPS to HT-29 cells (data not shown).

Effect of glucosamine on binding of IL-1β

Furthermore, we investigated the effect of glucosamine on binding of IL-1β to HT-29 cells using ^{125}I-IL-1β. HT-29 cells were washed three times with PBS, trypsinized, and then transferred to tubes and counted by γ-counter. Interestingly, ^{125}I-IL-1β binding was not affected by the incubation of HT-29 cells with glucosamine (0.1 ～ 10 mM, Fig.2), suggesting that impaired IL-8 production from HT-29 cells by glucosamine is not due to the effect on IL-1β-binding. Furthermore, we investigated whether glucosamine affects the expression of IL-1 receptor by flow cytometry. The IL-1β signaling cascade is initiated by its binding to the IL-1 receptor. Glucosamine had no effect on the expression of IL-1 type 1 receptor (data not shown).

These results suggest that glucosmaine did not essentially affect the LPS- and IL-1β-binding to HT-29 cells and the expression of LPS and IL-1β receptor on HT-29 cells.

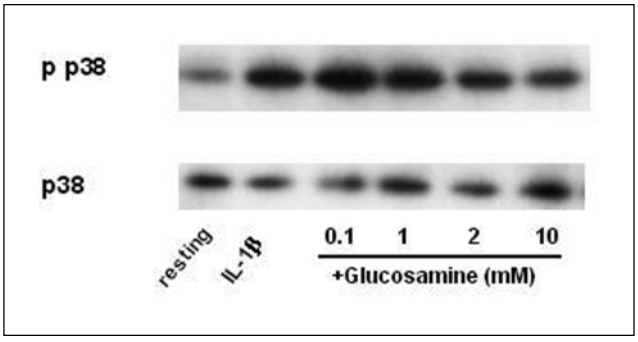

Fig.3 Effect of glucosamine on the p38 MAPK phosphorylation in HT-29 cells

HT-29 cells were pretreated with different concentrations of glucosamine for 30 min and than stimulated for 15 min in the presence of IL-1β (10 ng/ml), and were analyzed by Western blot using anti-phospho-p38 mAb. Western blot analysis using anti-p38 antibody was used as loading control.

Effect of glucosamine on the phosphorylation of p38 MAPK

To clarify the mechanism for the action of glucosamine, we investigated the signaling molecules that mediate IL-8 production. HT-29 cells were incubated with various concentration (0.1 ～ 10 mM) of glucosamine for 30 min before stimulation with IL-1β for 15 min. IL-1β-induced phosphorylation of p38 MAPK was determined by Western blot analysis. As shown in Fig.3, glucosamine inhibited IL-1β-induced phosphorylation of p38 MAPK in a dose-dependent manner, although almost the same amounts of p38 MAPK protein were analyzed in each sample.

These observations indicate that glucosamine inhibit IL-8 production from HT-29 cells by affecting the phosphorylation of p38 MAPK, downstream of the ligand binding to the receptors.

Conclusions

The gastrointestinal tract contains a unique lymphoid component designated the gut-associated lymphoid tissue (GALT). In addition, intestinal epithelial cell can also act as an immune cell, carrying out antigen presenting functions. In recent studies the colonic cancer cell lines HT-29, T84, and CaCo2 were reported to produce cytokines including IL-8, when stimulated with IL-1β, TNF-α and LPS[19,20]. IL-8 plays an important role in directing the sequential process of neutrophil rolling, adhesion, and transmigration in the inflamed microvasculature. Proinflammatory cytokines increased the expression of adhesion molecules on the endothelium and neutrophil. Consequently, neutrophils migrate

to the sites of gastrointestinal mucosal inflammation from peripheral blood. In fact, crypt abscess formation, as seen in UC, appears to be the result of neutrophil migration[22,23]. Moreover, in Japan, granulocyte adsorption apheresis therapy has been reported to show a remarkable therapeutic effect on active UC patients[24]. Furthermore, neutrophil elastase-specific inhibition has been reported to ameliorate murine dextran sulfate sodium (DSS)-induced colitis[22].

Recently, Hollenbach E. et al. investigated the impact of the p38 inhibitor SB203580 using a murine DSS-induced colitis model. They have reported that RIP-like interacting caspase-like apoptosis-regulatory protein kinase (RICK), p38 and NF-κB were strongly activated in the experimentally induced colitis, and these activations were drastically reduced by SB203580 treatment of the mice. These results suggest that p38, NF-κB and RICK are assumed to be a major mediator of inflammation in IBD[25]. In this study, we confirmed that glucosamine inhibits the IL-8 production from HT-29 cells by affecting the phosphorylation of p38 MAPK.

Previously, we showed that glucosamine suppressed neutrophil functions such as superoxide anion generation, phagocytosis, granule enzyme release and chemotaxis and also repressed the up-regulation of CD11b[15]. In preliminary experiments, we have examined the effect of glucosamine on DSS-induced colitis[26,27]. Glucosamine restored the clinical symptoms (based on the disease activity index), and suppressed the upregulation of CD11b on neutrophils (analyzed by flow cytometry) in DSS-induced colitis rats (data not shown). These observations suggest that glucosamine inhibit gastrointestinal inflammation in DSS-induced colitis though the inhibition of neutrophil activation.

Collectively, these observations provide the evidences that glucosamine attenuates the inflammatory response in the colonic cell line HT-29 by inhibiting the production of IL-8, and suppresses neutrophil activation in the colitis. Thus, glucosamine could be expected as a useful agent for the relief of inflammatory bowel diseases.

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