Carbohydrate Research 345 (2010) 1851–1855

Contents lists available at ScienceDirect

Carbohydrate Research

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

Phosphorylated glucosamine inhibits the inflammatory response in LPS-stimulated PMA-differentiated THP-1 cells

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ARTICLE INFO

Article history:
Received 27 January 2010
Received in revised form 3 June 2010
Accepted 15 June 2010
Available online 20 June 2010

Keywords:
THP-1 macrophage
Phosphorylated glucosamine
Anti-inflammation
MAPK

ABSTRACT

This study evaluated the effect of phosphorylated glucosamine (pGlc) on the regulation of cytokines involved in immunological activities. Changes in the inflammatory profiles of lipopolysaccharide (LPS)-stimulated phrobol 12-myristate 13-acetate (PMA)-differentiated THP-1 macrophage models were investigated following pGlc treatment. Treatment with pGlc inhibited the production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). In addition, pGlc suppressed the regulation of inflammatory mediators such as TNF-α, IL-1β, IL-6, inducible NO synthase (iNOS), and cyclooxygenase-2 (COX-2) in LPS-stimulated THP-1 macrophages. Furthermore, we confirmed that the LPS-stimulated transcription of MAP kinases in PMA-differentiated THP-1 macrophages was inhibited by pGlc. According to this study, pGlc can be considered as a potential anti-inflammatory agent.

1. Introduction

Inflammation reflects the pathological response to diseases such as atherosclerosis, cancer, diabetes, and Alzheimer's disease.[1] During the progression of inflammatory-related diseases, macrophages produce a high amount of pro-inflammatory cytokines, pro-inflammatory enzymes, and inflammatory mediators.[2] In many cell types, especially macrophages, lipopolysaccharide (LPS) acts as an endotoxin by its binding to the CD14/TLR4/MD2 receptor complex, which promotes the secretion of pro-inflammatory cytokines.[3] THP-1 cells, a human monocytic leukemia cell line, can be differentiated by phorbol 12-myristate 13-acetate (PMA) into macrophages due to morphological similarity. Differentiated THP-1 macrophages have been widely used as an in vitro model of human inflammatory disease since they allow the stimulation of the various genes involved in inflammation.[4,5] Inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) are important enzymes induced in macrophages by various inflammatory stimuli such as bacterial endotoxic LPS and cytokines, which themselves are involved in many inflammatory processes where they play an important role.[6] Therefore, the development of a cytokine inhibitor could protect against inflammation and inflammatory disease.

Glucosamine (Glc) is derived from chitin, which is a widely known therapeutic agent for the treatment of arthritis patients. *N*-Acetyl-glucosamine, glucosamine hydrochloride, and sulfated glucosamine have all been generally used as nutritional supplements.[7] It has been reported that sulfated glucosamine has positive effects on MMP and osteoblastic differentiation.[8] However, the biological activities of phosphorylated glucosamine are still poorly understood. A few studies reported that phosphorylated chitosan oligosaccharide inhibited the formation of calcium phosphate.[9] Therefore, we expect that phosphorylated glucosamine might contribute to several kinds of biological activities.

In this study, the anti-inflammatory effect of phosphorylated glucosamine (D-glucosamine-6-phosphate, pGlc, Fig. 1) in LPS-stimulated THP-1 macrophages was investigated by measuring the production and regulation of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, iNOS, and COX-2. Moreover, to understand the mechanism by which pGlc alters the inflammatory profile, we examined whether the inhibition of activated MAPK is critical for the anti-inflammatory function of pGlc.

2. Results

2.1. Effect of pGlc on THP-1 macrophage viability

The cytotoxicity of pGlc on the viability of THP-1 macrophages was investigated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. PMA-differentiated THP-1 macrophages were treated with or without pGlc at concentrations of 10, 50, 100, and 200 μg/mL. No concentration of pGlc exhibited any significant cytotoxicity (data not shown). Results obtained from MTT assay revealed that pGlc is a safe compound for in vitro cell culture experiments.

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doi:10.1016/j.carres.2010.06.006

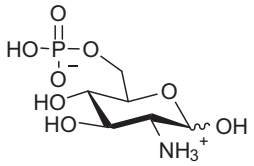

Figure 1. Structure of phosphorylated glucosamine (D-glucosamine-6-phosphate).

2.2. Effect of pGlc on the secretion and regulation of inflammatory cytokines

As a screening work for investigating the effect of pGlc on inflammatory action, anti-inflammatory effects of controls with glucosamine and pGlc on LPS-stimulated THP-1 macrophages were compared by ELISA (Fig. 2). THP-1 macrophages were co-cultured with various concentrations of the sample and then stimulated with LPS for 24 h. LPS stimulation of THP-1 macrophages induced the secretion of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6. However, pGlc treatment decreased secretion levels in the cultured media of LPS-stimulated THP-1 macrophages. Glucosamine exhibited no significant effect on pro-inflammatory cytokine production up to the concentration of 200 μg/mL, compared to pGlc (data not shown).

Next, we determined whether pGlc affects the transcriptional activation of TNF-α, IL-1β, and IL-6 using real-time PCR analysis (Fig. 3). The results show that the mRNA expression levels of TNF-α, IL-1β, and IL-6 were indeed increased by LPS stimulation, but then reduced by pGlc treatment, which is consistent with the results obtained during cytokine production. Therefore, pGlc prevented the production of TNF-α, IL-1β, and IL-6 by suppressing their gene expression in LPS-stimulated THP-1 macrophages.

The effect of pGlc on the regulation of cytokine mRNA and the protein expression in LPS-stimulated THP-1 macrophages were confirmed by RT-PCR and Western blot analysis, respectively (Fig. 4A and B). LPS stimulation upregulated the mRNA and protein levels of TNF-α, IL-1β, IL-6, COX-2, and iNOS, but pGlc treatment downregulated the cytokine levels depending on the concentration.

2.3. Activation of MAP kinase signaling pathways by pGlc

We next evaluated the activation of signal transduction by pGlc in relation to cytokine release. MAP kinases are mediators of important signaling pathways that control the synthesis and release of pro-inflammatory cytokines by activated macrophages during the inflammatory process.[10] THP-1 macrophages were co-cultured with various concentrations of pGlc for 1 h and then stimulated with LPS for 24 h. The phosphorylation of three MAP kinases, ERK, JNK, and p38 MAP kinase, was analyzed in THP-1 macrophages by Western blot analysis. It was found that phosphorylation of ERK, JNK, and p38 MAP kinase was rapidly induced by LPS stimulation (Fig. 5), but was suppressed by pGlc treatment. These results indicate that signal transduction by MAP kinases might be blocked by pGlc in activated macrophages.

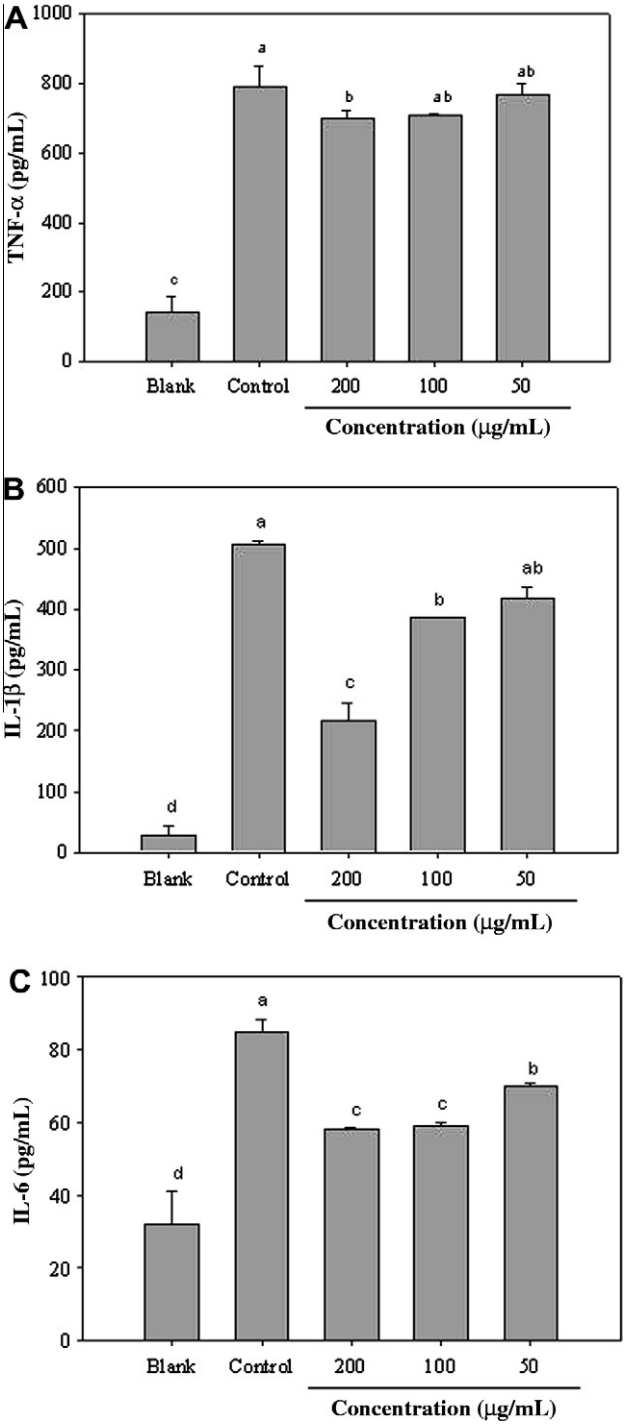

Figure 2. Effect of pGlc on pro-inflammatory cytokine release. The production of cytokines such as TNF-α (A), IL-1β (B), and IL-6 (C) was determined using ELISA. [a–d] Means with different letters in the same group are significantly different (p <0.05) by Duncan's multiple range test. Blank: −LPS, Control: +LPS.

3. Discussion

In the present study, we described the pro-inflammatory cytokine response in vitro in human macrophages to elucidate the mechanism of action of pGlc. Chronic inflammation is an early host immune response modulated by immune cells, especially macrophages and their cytokines.[11,12] Macrophages are highly activated during the inflammatory process by the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6.[13] These are major pro-inflammatory cytokines in immune cells and can cause chronic inflammatory diseases such as rheumatoid arthritis and atherosclerosis.[13–16] LPS is often used as the prototypical inflammatory stimulus due to its ability to initiate a range of pro-inflammatory mediators and induce the expression of inflammatory enzymes and cytokines.[17,18] Therefore, studying the inhibition of these inflammatory cytokines is considered key in the development of anti-inflammatory drugs. In our study, pGlc effectively inhibited the secretion of TNF-α, IL-1β, and IL-6 as well as downregulated