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The inhibitory mechanism of a novel cationic glucosamine derivative against MMP-2 and MMP-9 expressions

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ABSTRACT

A number of recent researches have demonstrated the therapeutic effects of glucosamine (Glc) in a range of human diseases including arthritis. For the first time, we identified that a novel Glc derivative having quaternized amino functionality (QAGlc) suppresses MMP-9 and MMP-2, gelatinases in HT1080, human fibrosarcoma cells at 40 μg/ml, following stimulation with PMA. Reporter gene assay results revealed that, the mechanism of suppression involves decreased transcriptional activation of MMP-9 and MMP-2 via transcription factors NF-κB and AP-1. However based on western blot results, QAGlc did not attenuate the nuclear translocation of both NF-κB and AP-1. Apparently, phorbol myristate acetate (PMA) stimulated expressions of ERK, JNK and p38 were altered in the presence of potent tumour inducer, phorbol myristate acetate QAGlc, suggesting their suppression also contributes to QAGlc-mediated inhibition of MMP-9 and MMP-2. Moreover, the ability of QAGlc to inhibit gelatinases was confirmed by its ability to act against invasiveness of HT1080 cells through extracellular matrix components.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent neutral endopeptidases that are collectively capable of degrading essentially all of the components of the extracellular matrix (ECM). The human MMP gene family consists of at least 18 structurally related members that fall into five classes according to their primary structure and substrate specificity: collagenases, gelatinases, stromelysins, membrane type (MT)-MMPs, and non-classified MMPs.[1] MMP expression is increased and strongly correlated with tumor invasiveness and poor prognosis reinforcing the concept that MMPs contribute to human cancer development.[2] Among the MMPs, gelatinases (MMP-2 and MMP-9) have been most consistently detected in malignant tissues and possible use of their inhibitors has been suggested in the future to augment treatment strategies in specific cancers.

MMPs are highly regulated at the levels of both gene expression and protein activation. Transcriptional regulation of MMP genes is frequently suggested to be mediated by an AP-1 regulatory element in their proximal promoter regions.[3] Studies on the promoter of MMP-9 have clearly identified that its transcription is mediated mainly via AP-1 transcription factor binding interactions. However, other reports on the promoter of MMP-9 suggest the involvement of NF-κB transcription factor for the activation of MMP-9.[4] Moreover, MMP-2 promoter has a number of potential cis-acting regulatory elements.[5] However, in recent years, NF-κB-dependent activation of MMP-2 has been reported.[6]

Even though NF-κB and AP-1 transcription factors are regulated by different mechanisms, they appear to be activated simultaneously by the same multitude of stimuli.[7] Indeed, the activation of mitogen activated protein kinases (MAPK) is often accompanied by the nuclear translocation of NF-κB, and many genes require the concomitant activation of AP-1 and NF-κB, suggesting that these transcription factors work cooperatively.[8] Persistent activation of MAPK in malignant cells can lead to enhanced induction of MMPs and this could lead to ECM and basement-membrane degradation allowing the cancer cells to invade into surrounding tissues and metastasize.[9]

The naturally occurring inhibitors of MMP activity (TIMPs) were the first compounds to be considered for clinical development. Among TIMPs, TIMP-1 forms preferential complexes with pro-MMP-9, whereas TIMP-2 and TIMP-4 exhibit high affinity for pro-MMP-2 exerting substrate specific effects.[10] However, the lack of effective methods of systemic gene delivery has limited the clinical utility of this approach. The rational chemical design of MMP inhibitors made possible the synthesis of compounds with specific inhibitory activities against the MMP subtypes that predominate in certain diseases, such as cancer and arthritis. Several effective MMP inhibitors have also been identified from natural sources. They are mainly plant origin such as resveratrol, theaflavins, catechins, curcumin and genistein.

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Glucosamine (Glc) salts constitute a new class of nutraceutical components due to their suggested beneficial roles in the human body.[11,12] The characteristic of Glc that is most important in the field of chemistry is this monomer has nucleophilic primary amino group. The reactivity of this amino group is convenient as they allow Glc derivatives to be generated with a range of facile chemistries or biochemistries. QAGlc, a novel Glc derivative was identified as a potent MMP-9 and MMP-2 inhibitor in HT1080, human fibrosarcoma cells in an effort to screen number of Glc derivatives having different functional groups. The results of this study present the alteration of transcription factors, AP-1 and NF-κB, and other genes accompany their activation in the presence of QAGlc.

Preparation of QAGlc was carried out according to Huang et al.[13] Resulting QAGlc was purified using a 100 Da molecular weight cut-off membrane (Spectrum Laboratories Inc. RanchonDominguez, CA) and a Dowex-50WX2 cation exchange resin (Dow Chemical Company (Michigan, USA) using modified phenol-sulfuric acid method.[14] The structure of purified QAGlc was determined by ^{1}H NMR, ^{13}C NMR spectroscopy (JNM-ECP-400 (400 MHz) spectrometer, JEOL, Japan), elemental (C, N, and H) analysis (Elementar Analysesysteme, Elementar Vario, EL, USA), and IR spectroscopy (Spectrum 2000 FT-IR spectrophotometer, Perkin-Elmer, USA). FT-IR (KBr, r, cm^{-1}): 3411 (OH), 2929, 2805 (CH), 1639 (CO), 1480 (Me), 1309 (CN), 1115, 1092, 1033 (pyranose) cm^{-1}; ^{1}H NMR (D_2O, 400 MHz, ppm): δ 5.4, 4.9 (1H, H-1_α, H-1_β), 3.2, 2.9 (1H, H-2_α, H-2_β), 3.4–3.9 (1H, H-3, 1H, H-4, 1H, H-5 and 2H, H_2-6), 4.7 (D_2O), 2.8 (2H, H_2-7 and 2H, H_2-9), 3.1 (9H, H_9-10); ^{13}C NMR (D_2O, 400 MHz, ppm) δ 89.2, 92.4 (C-1_α, C-1_β), 54.5, 56.1 (C-2_α, C-2_β), 69.3 (C-3), 76.1 (C-4), 71.4, 72.3 (C-5_α, C-5_β), 61.3 (C-6), 64.4 (C-7), 57.2 (C-8), 65.1 (C-9), 54.2 (3C,10-NMe); elemental analysis: C% (43.61), N% (8.52), H% (8.19).

Cells (HT1080) cultured in DMEM supplemented with 10% fetal bovine serum, were treated with different concentrations of QAGlc and non-toxic concentrations were used for the experiment. MMP-9 and MMP-2 activities in conditioned media following treatment with cells were assayed by gelatin zymography as described previously.[15] It was clearly observed that latent forms of MMP-9 (proMMP-9) and MMP-2 (proMMP-2) were converted into their active forms followed by treatment with phorbol myristate acetate (PMA), a potent tumor inducer (Fig. 1A). As depicted in Figure 1B, PMA (10 ng/ml) enhanced the MMP-9 and MMP-2 expressions by about 60% and 90%, respectively, compared to that of non-stimulated cells over the time. However, size and the intensity of lytic zones resulted due to gelatinolytic activities of MMP-9 and MMP-2 were greatly reduced in the presence of QAGlc and it clearly indicated that expression and activation of MMP-9 and MMP-2 in HT1080 cells were markedly inhibited in the presence of QAGlc. Moreover, inhibitory effect of QAGlc on MMP-9 and MMP-2 showed a concentration dependant pattern and at a concentration of 60 μg/ml QAGlc showed approximately 50% inhibition of MMP-9 led gelatinolytic activity. This activity was much clear than the effect of doxycycline (DOX), a tetracycline analogue we used in this study as the positive control to compare the inhibitory effects. Further, at the same concentration of QAGlc, about 62% inhibition of MMP-2 expression level was observed in the zymogram. However, Glc, the starting material of QAGlc did not exhibit any inhibitory activity against MMP-9 and MMP-2 at all concentrations employed.

To assess direct inhibitory activity against enzymes, conditioned media were treated with similar concentrations of doxycycline (DOX) and QAGlc and the reaction mixture was then zymographed according to the same above procedure.[15] In that experiment alterations in enzymatic activities of MMP-9 or

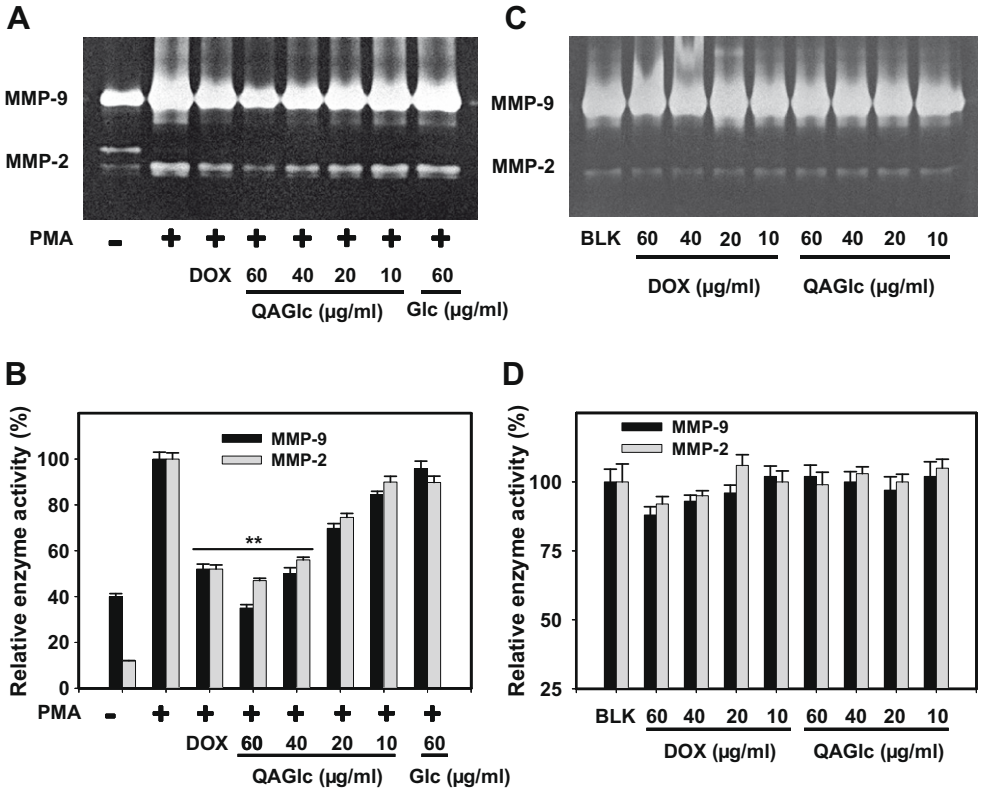

Figure 1. (A) MMP-9 and MMP-2 gelatinolytic activities in conditioned media of PMA stimulated HT1080 cells following treatment with QAGlc. The conditioned media was subjected to electrophoresis in a 10% (w/v) polyacrylamide gel impregnated with gelatin. Zymograms were developed in the presence of developing buffer, stained and the image of the gel was recorded using an image reader. Both MMP-9 and MMP-2 activities were significantly ($P < 0.01$) reduced by the positive control, doxycycline (DOX) and QAGlc at 40 and 60 μg/ml. (B) MMP-9 and MMP-2 activities as percentages in conditioned media of PMA stimulated HT1080 cells following treatment with QAGlc. (C) Direct inhibition of MMP-9 and MMP-2 gelatinolytic activities in conditioned media treated with QAGlc. Cells were stimulated with PMA and the conditioned media was treated with QAGlc. (D) MMP-9 and MMP-2 activities as percentages in conditioned media treated with QAGlc.