Inhibition of two bi-deoxyxygenated N-acetyl glucosamines against lipopolysaccharide-induced systemic inflammatory response in mice

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INTRODUCTION: N-acetyl glucosamine (NAG) is a natural amino sugar found in various human tissues with previously described anti-inflammatory effects. Various chemical modifications of NAG have been made to promote its biomedical applications. In this study, we investigated the in vivo and in vitro anti-inflammatory properties of two bi-deoxyxygenated NAG, BNAG1 (1,3-biodeoxy-N-acetyl-D-glucosamine or 2-Acetamido-1,2,3-trideoxy-D-glucose) and BNAG2 (4,6-biodeoxy-N-acetyl-D-glucosamine or 2-Acetamido-2,4,6-trideoxy-D-glucose) (Scheme 1).

METHODS: This study was approved by IACUC. Systemic inflammatory response to lipopolysaccharide (LPS) challenge in mice was induced with a single dose (10 mg/Kg, intraperitoneal injection) of LPS, 30 min after intravenous injection of NAG or BNAG1 or BNAG2 or the vehicle (saline). Pro-inflammatory cytokines IL-6 and TNF α concentrations in the serum from mice or culture medium from primary peritoneal macrophages were determined using the enzyme-linked immunosorbent assay (ELISA) kits. Leukocyte migration to the lungs and the peritoneal cavity were evaluated using a myeloperoxidase (MPO) kinetic-colorimetric assay and double-staining with Alexa Fluor® 568 phallodin and 4’, 6-diamidino-2-phenylindole (DAPI) which shows the actin cytoskeleton and cell nucleus, respectively. Data were expressed as mean ± SD. A student t-test (two-tailed) was performed to compare the difference between the selected two groups, and p<0.05 was considered significant.

RESULTS: LPS increased serum levels of IL-6 and TNF α, and all three tested compounds BNAG1, BNAG2 and NAG at the dose of 300 mg/kg were able to significantly decrease serum levels of IL-6 and TNF α in LPS-mice (Figs. 1A). Moreover, at a reduced dose of 200 mg/kg for the three compounds, BNAG1 showed significantly higher inhibitory activities against IL-6 and TNF α production (Figs. 1B) as well as the leukocyte migration to lungs (Fig. 2) and peritoneal cavity (Fig. 3), than either NAG or BNAG2 in LPS challenged mice. BNAG1 also revealed highest inhibition against IL-6 and TNF α production in LPS-stimulated primary peritoneal macrophages (data not shown).

DISCUSSION: BNAG1 and BNAG2 were reported for the first time as the bi-deoxyxygenated derivatives of NAG. It was demonstrated that BNAG1 had the highest activities among the three compounds against LPS induced inflammatory response both in mice and in vitro macrophage cultures. Further studies are needed to better clarify the exact mechanisms by which BNAG1 and BNAG2 mediate their effects.

SIGNIFICANCE/CLINICAL RELEVANCE: Inflammation is an exceedingly complex physiological pathway that underlies many human pathologies. The current findings implied potential application of these NAG derivatives, especially BNAG1 in the treatment of certain inflammation-related diseases such as joint damage, inflammatory bowel disease, autoimmune diseases, and viral respiratory infections.

![Scheme 1. The compounds studied in this project.](image)

![Fig. 1. The serum IL-6 and TNF α levels in mice. N=8](image)

![Fig. 2. MPO activities of the lung tissues from mice with various treatments.](image)

![Fig. 3. Leukocytes migration to the peritoneal cavity from mice with various treatments.](image)