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Effect of ether glycerol lipids on interleukin-1β release and experimental autoimmune encephalomyelitis

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ABSTRACT

We have assessed the effect of two ether glycerol lipids, **77-6** ((2*S*, 3*R*)-4-(Tetradecyloxy)-2-amino-1,3-butanediol) and **56-5** ((*S*)-2-Amino-3-*O*-hexadecyl-1-propanol), which are substrates for sphingosine kinases, on inflammatory responses. Treatment of differentiated U937 macrophage-like cells with **77-6** but not **56-5** enhanced IL-1β release; either alone or in the presence of LPS. The stimulatory effect of sphingosine or **77-6** on LPS-stimulated IL-1β release was reduced by pretreatment of cells with the caspase-1 inhibitor, Ac-YVAD-CHO, thereby indicating a role for the inflammasome. The enhancement of LPS-stimulated IL-1β release in response to sphingosine, but not **77-6**, was reduced by pretreatment of cells with the cathepsin B inhibitor, CA074Me, indicating a role for lysosomal destabilization in the effect of sphingosine. Administration of **56-5** to mice increased disease progression in an experimental autoimmune encephalomyelitis model and this was associated with a considerable increase in the infiltration of $CD4^+$ T-cells, $CD11b^+$ monocytes and $F4/80^+$ macrophages in the spinal cord. **56-5** and **77-6** were without effect on the degradation of myc-tagged sphingosine 1-phosphate 1 receptor in CCL39 cells. Therefore, the effect of **56-5** on EAE disease progression is likely to be independent of the inflammasome or the sphingosine 1-phosphate 1 receptor. However, **56-5** is chemically similar to platelet activating factor and the exacerbation of EAE disease progression might be linked to platelet activating factor receptor signaling.

1. Introduction

Innate immunity to pathogens uses pathogen-associated molecular patterns (PAMPs) to increase IL-1β and IL-18 release from inflammatory cells (Schroder and Tschopp, 2010). Lipopolysaccharide (LPS) is an example of a PAMP that can bind to toll-like receptors (TLR) to initiate innate immune responses (Takeuchi and Akira, 2010). In addition, during sterile inflammation, danger-associated molecular patterns (DAMPs) can be released from dead cells to promote innate immune responses via TLR-dependent pathways (Chen and Nunez, 2010). The NLRP (NOD-like receptor family, pyrin domain containing) inflammasome senses DAMPs, resulting in the binding of apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) to the inflammasome. This, in turn, promotes assembly and activation of caspase-1, which cleaves pro-IL-1β to produce bioactive IL-1β which is then released from cells (Brough and Rothwell, 2007). DAMPs might also be released from the damage to lysosomal membranes, which is regulated by lysosomal proteases, such as cathepsin B (Hornung and Latz, 2010). Recently, Luheshi et al. (2012) demonstrated that sphingosine and the sphingosine analogue, FTY720, promote the NLRP3-dependent release of IL-1β from LPS-stimulated macrophages, as this was not evident in macrophages derived from *Nlrp3*$^{-/-}$ mice. The effect of sphingosine on IL-1β release involves the protein phosphatase 2A and/or protein phosphatase 1 (PP2A/PP1). Thus, the PP2A/PP1 inhibitors okadaic acid and calyculin A prevented sphingosine-induced IL-1β release from macrophages (Luheshi et al., 2012). Moreover, sphingosine-induced IL-1β release was unaffected by the cathepsin B inhibitor, CA074Me, or the pan-cysteine protease inhibitor, E64 (Luheshi et al., 2012).

Abbreviations: DAMPs, danger associated molecular patterns; DMEM, dulbeccos modified eagles medium; EAE, experimental autoimmune encephalomyelitis; ERK, extracellular signal regulated kinase; LPS, lipopolysaccharide; NLRP3, NOD like receptor familypyrin domain containing 3; IFNγ, interferon gamma; IL-1β, interleukin-1beta; PAF, platelet activating factor; PAMPs, pathogen-associated molecular patterns; PP2A, protein phosphatase 2A; PP1, protein phosphatase 1; SK, sphingosine kinase; S1P, sphingosine 1-phosphate; $S1P_1$, sphingosine 1-phosphate receptor-1; THT, helper cells; TLR, toll-like receptors.

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The possibility that the effect of sphingosine on NLRP3-dependent release of IL-1β is mediated through metabolites of sphingosine was excluded based on the finding that ceramide, which is formed by acylation of sphingosine (catalysed by ceramidase and/or ceramide synthase) had no effect on IL-1β release. Moreover, much higher concentrations of sphingosine 1-phosphate (compared with sphingosine), which is produced by phosphorylation of sphingosine by the action of sphingosine kinase (two isoforms termed SK1 and SK2), were required in order to stimulate IL-1β release (Luheshi et al., 2012). These findings can possibly be explained by evidence showing that sphingosine potently binds to the acidic leucine rich nuclear phosphoprotein-32A (ANP32A) to activate PP2A (Habrukowich et al., 2010), and that this might also underlie the ability of sphingosine to inhibit cell growth and induce apoptosis of mammalian cells (Ohta et al., 1995; Nava et al., 2000) and to reduce tumour growth in vivo (Kohno et al., 2006).

Multiple sclerosis is an autoimmune inflammatory demyelinating disease involving reactive T-lymphocytes. There is also a strong prognostic relationship between IL-1β levels and disease progression and mutation of the *Nlrp3* gene is associated with MS-like lesions (Compeyrot-Lacassagne et al., 2009; Dodé et al., 2002). In addition, mice lacking the gene encoding NLRP3 (*Nlrp3*$^{-/-}$) develop significantly milder symptoms in an experimental autoimmune encephalomyelitis (EAE) model and exhibit a reduction in IFNγ- and IL-17-expressing TH cells in peripheral lymphoid tissues and the spinal cord (Gris et al., 2010; Inoue et al., 2012). IL-1β also induces activation of microglial cells, which stimulate T-lymphocytes with self-antigen during EAE development. In addition, IL-1 receptor deficient mice develop milder EAE and reduced TH17 cells (Sutton et al., 2006). There is also a relationship between multiple sclerosis, NLRP3 inflammasome and the sphingosine analogue, FTY720. Indeed, FTY720 has been shown to activate PP2A, to stimulate IL-β release via an NLRP3-dependent mechanism in macrophages (Luheshi et al., 2012) and to modulate the immune response by preventing egress of T-lymphocytes from lymph nodes (Hla and Brinkmann, 2011). FTY720 is licenced for oral treatment of relapsing multiple sclerosis under the trade name Gilenya™. FTY720 is a prodrug, which is phosphorylated by SK2 and is a functional antagonist of sphingosine 1-phosphate receptor 1 ($S1P_1$), causing this receptor to be degraded by the proteasome and removed from T-lymphocytes (Hla & Brinkmann, 2011). Since T-lymphocytes use an S1P gradient to egress from lymph nodes, FTY720 is able to prevent this by creating $S1P_1$ null T-lymphocytes that do not respond to the S1P gradient. This traps the T-lymphocytes in lymph nodes, thereby preventing their action on the CNS in multiple sclerosis.

In the current study, we have assessed whether ether glycerol lipids can affect inflammasome-dependent IL-1β release from macrophages and modify disease progression in the EAE model. The rationale for this was three-fold. First, there is structural similarity of the ether glycerol lipids with sphingosine and therefore these molecules might act in a similar manner as sphingosine to modulate inflammasome-dependent IL-1β release. Second, if modulation of inflammasome-dependent IL-1β release is evident, then these ether glycerol lipids might serve as a starting point for the synthesis of potent inhibitors of the inflammasome that can be used therapeutically to abrogate inflammatory diseases. Alternatively, the synthesis of potent inflammasome activators could be used promote innate immunity against invading pathogens. Third, previous studies using 1-*O*-hexadecyl-2-desoxy-2-amino-*sn*-glycerol (which is **56-5**) have demonstrated its phosphorylation by SK1 (K_m = 3.8 μM, compared with K_m = 15.7 μM for sphingosine (sphing-4-enine) (Gijsbers et al., 2002). Indeed, we show here that ether glycerol lipids are substrates for SK1 and SK2, and that **77-6** is a powerful stimulator of IL-1β release from macrophages. However, **56-5** which fails to stimulate IL-1β release in U937 macrophage-like cells, increases disease progression in the EAE model. This action of **56-5** might be related to its chemical similarity with platelet activating factor, which also has a role in multiple sclerosis (Kihara et al., 2005).

2. Results and discussion

***77-6** and **56-5** are substrates for both SK1 and SK2*: We assessed whether several ether glycerol lipids (**56-2**, **56-3**, **56-4**, **56-5**, **77-5** and **77-6**; Fig. 1) and a sphingosine analogue (**67-622**, Fig. 1) inhibit SK1 and SK2 activity and/or function as substrates for these enzymes (Table 1). The replacement of the azide group in **56-4** a compound which inhibited SK2 activity, with an —NH_2 group produced compound **56-5** ((*S*)-2-Amino-3-*O*-hexadecyl-1-propanol or 1-*O*-hexadecyl-2-desoxy-2-amino-*sn*-glycerol), which was a substrate for SK2 (Table 1). **56-5** was also a weak substrate for SK1, being ~5 times less effective than for SK2 (Table 1). Similarly, replacement of the azide in **77-5** with an —NH_2 group produced compound **77-6** ((2*S*, 3*R*)-4-(tetradecyloxy)-2-amino-1,3-butanediol), which was a substrate for SK2; this being ~3 times as effective compared with **56-5** (Table 1). Therefore, reduction in the alkyl chain length from C16 to C14 and introduction of the 3-hydroxyl group, enhanced substrate utilization by SK2. Indeed, the 3-hydroxyl group of the naturally occurring substrate of SK2, sphingosine, might H-bond with D308 to orientate the 1-hydroxyl group for phosphorylation by the enzyme. **77-6** was also a preferred substrate for SK1 compared with **56-5**. **56-5** and **77-6** bear chemical resemblance with sphingoid bases and this might therefore provide explanation for why these molecules can function as substrates for both SK1 and SK2. Indeed, the effect of carbon alkyl chain length on the efficiency of phosphorylation

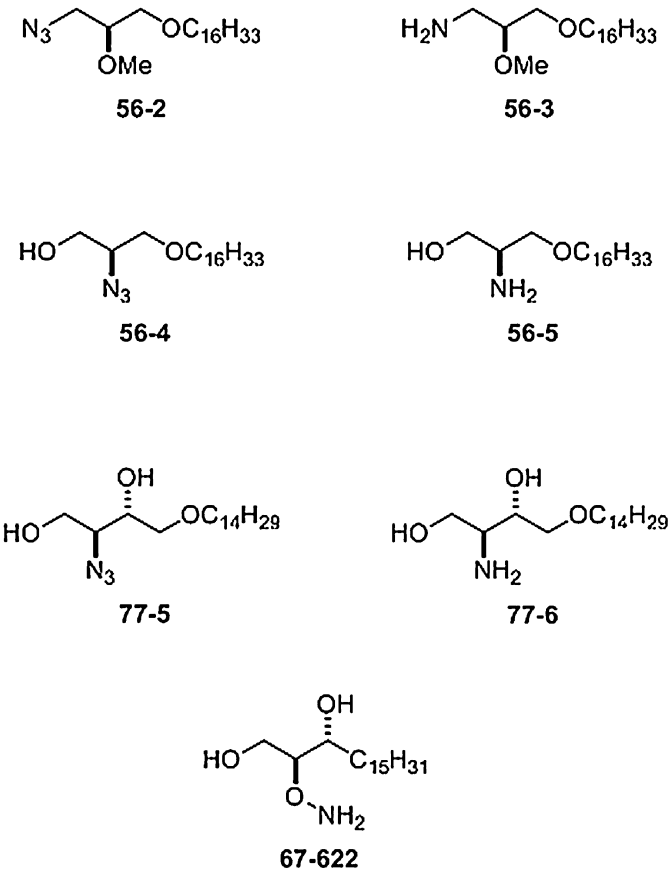

Fig. 1. Chemical structures.