Abstract

Humans face a constant threat from pathogens like influenza varieties H1N1, H5N1, and others and there is a need to prevent these from epidemics. The pathogens depend on successful colonization of the host in order to reproduce and multiply. Sialidases are known as neuraminidases are a group of enzymes, the most abundant of these being the exo-sialidases that can catalyze the cleavage of sialic acids from carbohydrates, glycoproteins or glycolipids. Sialidases have been thoroughly studied since their discovery 75 years ago and their occurrence in bacteria and viruses is widespread. They are found in diverse virus families and bacteria and other microbes. Moreover, sialic acids serve as a receptor for various pathogens. This allows bacteria like H1N1 or other influenza viruses, to enter the host cell. There is a need to block sialidases as they release sialic acid that serves as nutrition for the microbes and as well allows them to bind and invade the host cell where they can proliferate. This makes sialidases an interesting target to control pathogenic activity.

Sialidase inhibition is seen in many infectious and autoimmune diseases. Since it is safer, it has the potential of being directly tested on humans without side effects and could have a potential role in mitigating the pathogens that cause a burden on the Public health system.

Keywords: Sialidases; CD33; Sialic acid; Viruses; Bacteria; Influenza

Introduction

Sialidases are also known as neuraminidases, and they are enzymes, the majority of which are exo-Sialidases that catalyze the cleavage of sialic acids from glycoproteins or glycolipids [1,2]. Sialidase activity is seen in many infective and autoimmune diseases [3-5]. Sialidases are involved in pathways like immunosuppression and rapidly multiply within the host. Sialidase activity is seen in cancer and also in biofilm formation [6,7]. Neu3, a sialidase, is upregulated in these diseases [8,9]. Defects in the sialidase activity of the brain have been shown to have a role in different psychiatric and neurological disorders like epilepsy, alcoholism, schizophrenia and severe depression [10]. Sialic acids are found at terminal positions of many surface-exposed glycoconjugates and are vulnerable to cleavage by sialidases [2,11].

Sialidases are not only expressed in humans but also in bacteria. Viruses, as well as fungi species and are involved in the modulation of molecules linked to biological processes [12,13]. Sialic acid is synthesized de novo or obtained exogenously. Many pathogens camouflage their surface molecules, polysaccharides and LPS with sialic acid, which makes their entry into host cell surfaces easier. This action helps them evade the immune response by the host immune system [14,15]. Bacteria acquire sialic acid by synthesizing it or acquire it from the environment [16,17]. Many pathogens use a sialidase to release sialic acid from the host sialoglycoconjugates [18]. Other bacteria that cannot secrete a sialidase are dependent on host-derived sialic acid [19].

Chen et al. in their work with intestinal sepsis came to the conclusion that inflammatory response is exacerbated by bacterial sialidases, keeping immune responses in check when ‘host’ cells are damaged. Inhibiting sialidase activity leads to a substantial reduction of the inflammatory response and increases in subsequent morbidity [20]. The cleavage of human sialic acids from glycoproteins or glycolipids by pathogenic sialidases is involved in many infectious, chronic as well as autoimmune diseases. The influenza viral drugs Tamiflu and Relenza inhibit the influenza virus sialidase, which is required for viral replication from infected cells.

Our work is an extension of the previous work that Metadichol® is a food-based nanoemulsion of long-chain based alcohols inhibits H1N1 and other viruses including Zika and Ebola [21-23]. To understand the mechanism of the antiviral, antibacterial property of Metadichol®, we recently showed that Metadichol increases CD33 expression by up to 400 fold at 100 picograms per ml in Umbilical cord cells and CD33 binds to related Siglecs [24].

In this study, THP-1 cells were used as an in-vitro model for human monocytes for its response to LPS induced sialidase production. LPS is a surface component of gram-negative bacteria that plays a critical role in mediating inflammation and inducing cells to secrete pro-inflammatory cytokines.
Materials and Methods

Thp-1 (ATCC® TIB-202™) cells were procured from ATCC, USA. RPMI media, Fetal Bovine Serum (FBS) and PenStrep were obtained from Life Technologies, USA. 4-methylumbelliferyl-α-N-acetyl-D-neuraminic acid, Phorbol 12-myristate 13-acetate (PMA), L-glutamine, β-mercaptoethanol, PMA and phenyl methyl sulphonyl chloride (PMSF) were all obtained from Sigma Aldrich, USA.

Preparation of test samples

Metadichol (5 mg/ml) stock was diluted to obtain desired concentrations of 0.001, 0.1, 1, 100, 1000 ng/ml test solutions.

Cell lines and treatment

Cell culture: THP-1, a promonocytic cell line was obtained from the ATCC. Cells were cultured in T25 cm² flask with RPMI-1640 supplemented with 10% inactivated fetal bovine serum, 50 µM 2-mercaptoethanol, 2 mM L-Glutamine and penicillin and streptomycin (100 IU/ml) in a humidified atmosphere of 5% CO₂ at 37°C until confluent.

Treatment to determine the effects of a sample on sialidase activity: The cells were aspirated from the 80% confluence culture flask and centrifuged at 1500 rpm for 5 mins. The cell pellet was then resuspended in 1mL of RPMI complete media and counted conventionally using Hemocytometer. The cells (5 × 10⁶) were incubated with PMA (10 ng/ml) in separate dishes to differentiate THP1 cells. To determine the sialidase activity, THP-1 cells were pretreated for 1hr with Metadichol at various concentrations prepared in culture media without FBS and Prednisone at 100 and 1000 ng/mL as a positive control followed by 24 hr LPS (1 µg/ml) stimulation. Post incubation, the cells are carried over to determine the sialidase activity. The sialidase activity was found to be maximum at 16-hour time point.

Determination of Sialidase activity: Cells were washed with phosphate-buffered saline and resuspended in ice-cold buffer containing 0.25 M sucrose, 1 mM EDTA, and 0.2 mM phenylmethylsulphonyl fluoride. The cell suspension was sonicated on ice for 15 s on a low setting (6% amplitude) (VibracellTM; Sonics and Materials Inc., Newtown, CT) followed by centrifugation at 25,000 g for 15 min at 4°C. The resulting supernatant was used to determine the lysosomal sialidase activity. Protein quantification of the supernatant was performed using the Bio-Rad protein determination kit as described above. For the determination of lysosomal sialidase activity, 200 µg of total protein was mixed with 40 nmol of 4-methylumbelliferyl-α-N-acetyl-D-neuraminic acid (Sigma), the lysosomal sialidase-specific substrate, 10 µmol sodium acetate buffer, pH 4.6, and 200 µg of bovine serum albumin in a total volume of 200 µl. The sialidase reaction was allowed to proceed for 1 h at 37°C and was terminated by the addition of 0.25 M glycine NaOH, pH 10.4. Released 4-methylumbelliferyl-N-acetyl-D-neuraminic acid was measured fluorometrically (Synergy 2 multi-mode microplate reader) at an excitation wavelength of 365 nm and an emission wavelength of 448 nm.

Results and Discussion

The results show the sialidase activity assessed by the isolation of whole protein. With Metadichol the relative sialidase activity/mg of protein at 1 ng/ml was found to be 0.79 compared to LPS control. Prednisone shows no activity below 100 nanograms per ml and Metadichol shows activity at 1 picogram per ml (0.001 ng/ml) (Tables 1 and 2). The sialidase activity was found to be higher at 16 hour time point post LPS (1 µg/ml) treatment. Sialidase activity declined to post 16 hrs.

Table 1: Relative sialidase activity/mg of protein (Metadichol).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentrations</th>
<th>Mean Relative sialidase activity/mg of protein (Time points) (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media control</td>
<td>0</td>
<td>0.12 0.04 0.03 0.03</td>
</tr>
<tr>
<td>LPS (1 µg/ml)</td>
<td>LPS</td>
<td>1.00 1.00 1.00 1.00</td>
</tr>
<tr>
<td>Metadichol+LPS (1 µg/ml)</td>
<td>0.001 ng/ml+LPS</td>
<td>0.91 0.91 0.95 0.92</td>
</tr>
<tr>
<td></td>
<td>0.1 ng/ml+PS</td>
<td>0.87 0.87 0.90 0.87</td>
</tr>
<tr>
<td></td>
<td>1 ng/ml+LPS</td>
<td>0.81 0.80 0.83 0.79</td>
</tr>
</tbody>
</table>

Table 2: Relative sialidase activity/mg of protein (Prednisone).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentrations</th>
<th>Mean Relative activity/mg (n=2)</th>
<th>sialidase activity (Time)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media control</td>
<td>0</td>
<td>0.12 0.04 0.03 0.03</td>
<td></td>
</tr>
<tr>
<td>LPS (1 µg/ml)</td>
<td>LPS</td>
<td>1.00 1.00 1.00 1.00</td>
<td></td>
</tr>
<tr>
<td>Prednisone+LPS (Positive Control)+LPS (1 µg/ml)</td>
<td>0.1 µg/mL+LPS</td>
<td>0.87 0.86 0.82 0.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 µg/mL+LPS</td>
<td>0.68 0.69 0.68 0.67</td>
<td></td>
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</table>
We could not compare activity at higher concentration with Metadichol as it is toxic to the cancer cell lines. We have documented that pancreatic cancer cell line, express klotho genes on Metadichol treatment at picogram levels as Klotho gene blocks cancer cell growth [25]. Metadichol increases CD33 expression of 400 fold. CD33 binds to sialic acid so any excess sialic is soaked up by CD33 removing the concentration is elevated when compared with non-diabetic subjects [31]. Sialic acid is the risk factors for vascular disease, blood lipids, and lipoprotein [32,33].

We have shown that Metadichol modulates biomarkers like hypertension, Lipid profiles, sugar levels in diabetes (Type 1 and 2) and in addition to other pathways CD33 and inhibition of Sialidases have a role in mitigating the biomarkers of these diseases [34].

Metadichol® is derived from food-based ingredients with an LD50 of 5000 mg/kilo [35]. It has no toxic effects on humans and can directly be tested in human subjects in mitigating infectious and other chronic diseases [36-38].

Conclusion

The inhibition of sialidases by Metadichol is important. Tamiflu and Relenza by inhibiting sialidases in influenza virus prevent its spread to normal cells from infected cells. This property has utility in treatment of sepsis infection as well in other viral diseases. Metadichol is the first known nutraceutical to inhibit sialidases and would allow researchers to study in detail the mechanism of how viruses that threaten mankind can be controlled without the need to worry about toxic effects of drugs.

References