Journal of Stem Cell Research & Therapy

Raghavan et al., J Stem Cell Res Ther 2019, 9:4
DOI: 10.4172/2157-7633.1000449

Research Article Open Access

Metadichol® a Novel Sialidase Inhibitor

Raghavan PR[1*], Yogisha S[2], Anand S[2] and Purushotham GV[2]

[1]*Nanorx Inc, PO Box 131, Chappaqua, NY, 10514, USA*

[2]*Skanda Life Sciences, R and D Center, Bangalore, India*

[*]**Corresponding authors:** Raghavan PR, Nanorx Inc, PO Box 131, Chappaqua, NY, 10514, USA, E-mail: raghavan@nanorxinc.com

Received date: April 12, 2019; **Accepted date:** April 30, 2019; **Published date:** May 10, 2019

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.

Metadichol® is nanoemulsion of long-chain lipid alcohols derived from food ingredients. In rats, it has an LD50 of 5000 mg/kilo and its ingredients are present in many foods we consume on a daily basis. It has antiviral and antibacterial and anti-parasitic properties. We studied inhibition of Sialidases by inducing it with Lipopolysaccharide (LPS) using THP1 cells. Metadichol showed inhibition at 1 picogram per ml to 1 nanogram per/ml. Compared to Prednisone. It is 100 times more active. Previous studies on Metadichol® showed that it is toxic to cancer cells at higher concentrations.

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 a burden on the Public health system.

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

Introduction

Sialidases are also known as neuraminidases are enzymes, the majority of which are exo-Sialidases that catalyze the cleavage of terminal sialic acids from glycoproteins or glycolipids [1,2]. Sialidase activity is seen in many infectious and also 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® 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 cm^2 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% CO_2 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 at16-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. In the case of Prednisone (Figures 1 and 2) relative sialidase activity at 1000 ng/ml treatment was 0.67 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.

Sample	Concentrations	Mean Relative sialidase activity/mg of protein (Time points) (n=2)			
		4 hr	8 hr	16 hr	24 hr
Media control	0	0.12	0.04	0.03	0.03
LPS (1 µg/ml)	LPS	1.00	1.00	1.00	1.00
Metadichol+LPS (1 µg/ml)	0.001 ng/ml+LPS	0.91	0.91	0.95	0.92
	0.1 ng/ml+PS	0.87	0.87	0.90	0.87
	1 ng/ml+LPS	0.81	0.80	0.83	0.79

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

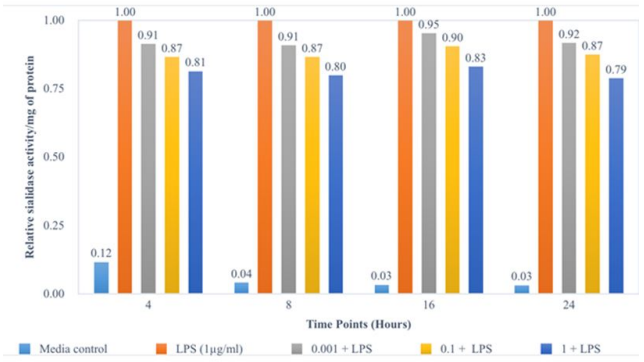

Figure 1: Representation of relative sialidase activity/mg of protein (Metadichol).

Sample	Concentrations	Mean Relative sialidase activity/mg of protein (Time points) (n=2)			
		4 hr	8 hr	16 hr	24 hr
Media control	0	0.12	0.04	0.03	0.03
LPS (1 µg/ml)	LPS	1.00	1.00	1.00	1.00
Prednisone (Positive Control)+LPS (1 µg/ml)	0.1 µg/mL+LPS	0.87	0.86	0.82	0.81
	1 µg/mL+LPS	0.68	0.69	0.68	0.67

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

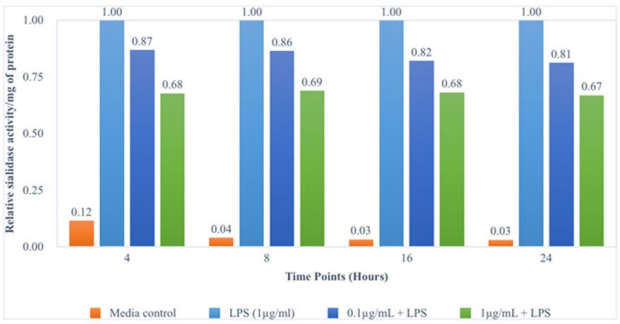

Figure 2: Representation of Relative sialidase activity/mg of protein (Prednisone).

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 access to pathological agents to use it either a source of energy or to circumvent the immune response from the host. Viruses and bacteria use sialic acid as a "mimicry'' to enter hosts cells then the increased CD33 and also sialidase downregulation plays a key role in the documented antimicrobial activity of Metadichol [24,26,27]

Sialic acid concentration is strongly related to microvascular complications in type I diabetes [28-30]. In type II diabetes, Sialic acid 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

1. Varki A (1992) Diversity in the sialic acids. Glycobiol 2: 25-40. [PubMed]
2. Schauer R, Kamerling JP (1997) Chemistry, biochemistry, and biology of sialic acids. Glycoproteins II. Elsevier Science AV, Amsterdam, Netherlands.
3. Bouchet V1, Hood DW, Li J, Brisson JR, Randle GA, et al. (2003) Host-derived sialic acid is incorporated into Haemophilus influenzae lipopolysaccharide and is a major virulence factor in experimental otitis media. Proc Natl Acad Sci USA 100: 8898-8903. [PubMed]
4. Tribulatti MV, Mucci J, Van Rooijen N, Leguizamon MS, Campetella O (2005) The trans-sialidase from Trypanosoma cruzi induces thrombocytopenia during acute Chagas' disease by reducing the platelet sialic acid contents. Infect Immun 73: 201-207. [PubMed]
5. Lanotte P, Watt S, Mereghetti L, Dartiguelongue N, Rastegar-Lari A, et al. (2004) Genetic features of Pseudomonas aeruginosa isolated from cystic fibrosis patients compared with those isolated from other origins. J Med Microbiol 53: 73-81. [PubMed]
6. Sawada M1, Moriya S, Saito S, Shineha R, Satomi S, et al. (2002) Reduced sialidase expression in highly metastatic variants of mouse colon adenocarcinoma 26 and retardation of their metastatic ability by sialidase overexpression. Int J Cancer 97: 180-185. [PubMed]
7. Parker D, Soong G, Planet P, Brower J, Ratner AJ, et al. (2009) The NanA neuraminidase of Streptococcus pneumoniae is involved in biofilm formation. Infect Immun 77: 3722-3730. [PubMed]
8. Moncla BJ, Braham P, Hillier SL (1990) Sialidase (neuraminidase) activity among gram-negative anaerobic and capnophilic bacteria. J Clin Microbiol 28: 422-425. [PubMed]
9. Sedlacek HH, Seiler FR, Schwick HG (1997) Neuraminidase and tumor immunotherapy. Klinische Wochenschrift 55: 199-214.
10. Boyzo A, Ayala J, Gutierrez R, Hernandez RJ (2003) Neuraminidase activity in different regions of the seizing epileptic and nonepileptic brain. Brain Res 964: 211-217. [PubMed]
11. Achyuthan K, Achyuthan A (2001) Comparative enzymology, biochemistry and pathophysiology of human exo-sialidases (neuraminidases). Comp Biochem Physiol B Biochem Mol Biol 129: 29-64. [PubMed]
12. Shaknovich EA, King SJ, Weiser JN (2002) Neuraminidase expressed by Streptococcus pneumoniae desialates the lipopolysaccharide of Neisseria meningitides and Haemophilus influenza: a paradigm for interbacterial competition among pathogens of the human respiratory tract. Infect Immun 70: 7161-7164. [PubMed]
13. Monti E, Preti A, Venerando B, Borsani G (2002) Recent development in mammalian sialidase molecular biology. Neurochem Res 27: 649-663. [PubMed]
14. Bonten E, van der Spoel A, Fornerod M, Grosveld G, d'Azzo A (1996) Characterization of human lysosomal neuraminidase defines the molecular basis of the metabolic storage disorder sialidosis. Genes Dev 10: 3156-3169. [PubMed]
15. Severi E, Hood WD, Thomas HG (2007) Sialic acid utilization by bacterial pathogens. Microbiology 153: 2817-2822. [PubMed]
16. Vimr E, Lichtensteiger C, Steenbergen S (2000) Sialic acid metabolism's dual function in Haemophilus influenzae. Mol Microbiol 36: 1113-1123. [PubMed]
17. Vimr ER, Kalivoda KA, Deszo EL, Steenbergen SM (2004) Diversity of microbial sialic acid metabolism. Micro Mol Biol Rev 68: 132-153. [PubMed]
18. Corfield T (1992) Bacterial sialidases-roles in pathogenicity and nutrition. Glycobiol 2: 509-521. [PubMed]
19. Hood DW, Cox AD, Gilbert M, Makepeace K, Walsh S, et al. (2001) Identification of a lipopolysaccharide alpha-2,3-sialyltransferase from Haemophilus influenza. Mol Microbiol 39: 341-350. [PubMed]
20. Chen GY, Chen X, King S, Cavassani KA, Cheng J, et al. (2011) Amelioration of sepsis by inhibiting sialidase-mediated disruption of the CD24-SiglecG interaction. Nat Biotechnol 29: 428-435. [PubMed]
21. Raghavan PR (2014) US Patents: 8,722,093.
22. Raghavan PR (2015) US Patents 9,034,383 and 9,006,292.
23. Raghavan PR (2016) Inhibition of dengue and other enveloped viruses by Metadichol®, a novel nanoemulsion lipid. J Healing Outcomes 8: 19-25.
24. Angata T, Varki A (2015) Siglec interactions with pathogens. Glycoscience: Biology and Medicine, Springer, Tokyo, Japan. [PubMed]

25. Raghavan PR (2018) Metadichol® a novel agonist of the anti-aging klotho gene in cancer cell lines. J Cancer Sci Ther 10: 351-357.
26. Raghavan PR (2017) Metadichol® and MRSA Infections: A Case Report. J Infect Dis Ther 5: 2.
27. Raghavan PR (2016) In vitro Inhibition of Zika Virus by Metadichol®: A novel nano emulsion lipid. J Immunol Tech Infect Dis 5: 4.
28. Crook MA, Tutt P, Simpson H, Pickup JC (1993) Serum sialic acid and acute phase proteins in type 1 and type 2 diabetes mellitus. Clin Chim Acta 219: 131-138. [PubMed]
29. Crook MA, Pickup JC, Lumb PJ, Giorgino F, Webb DJ, et al. (2001) Relationship between plasma sialic acid concentration and microvascular and macrovascular complications in type 1 diabetes. Diabetes Care 24: 316-322. [PubMed]
30. Lindberg G, Rastam L, Gullberg B, Eklund GA, Tornberg S (1991) Serum sialic acid concentration and smoking: A population-based study. BMJ 303: 1306-1307. [PubMed]
31. Rajappa M, Ikkruthi S, Nandeesha H, Satheesh S, Sundar I, et al. (2013) Relationship of raised serum total and protein-bound sialic acid levels with hyperinsulinemia and indices of insulin sensitivity and insulin resistance in non-diabetic normotensive obese subjects Diabetes and Metabolic Syndrome. Clin Res Rev 7: 17-19. [PubMed]
32. Wakabayashi I, Sakamoto K, Yoshimoto S, Masui H (1992) Relation of serum sialic acid to lipid concentrations. BMJ 305: 562-563. [PubMed]
33. Kario K, Matsuo T, Imiya M, Kayaba K, Kuroda T, et al. (1994) Close relation between lipoprotein (a) levels and atherothrombotic disease in Japanese subjects 475 years of age. Am J Cardio 73: 1187-1190. [PubMed]
34. Aleman CL, Puig MN, Elías EC, Ortega CH, Guerra IR, et al. (1995) Carcinogenicity of policosanol in mice: An 18-month study. Food Chem Toxicol 33: 573-578. [PubMed]
35. Raghavan PR (2016) Metadichol and type 2 diabetes a case report. J Sci Heal Outco 8: 5-10.
36. Raghavan PR (2010) Case report of type 1: Diabetes. J Sci Heal Outco 2: 24.
37. Raghavan PR (2017) Rheumatoid arthritis and osteoporosis: A case study. J Arthritis 6: 240.
38. Raghavan PR (2017) Systolic and diastolic BP control in metabolic syndrome patients with Metadichol® a novel nanoemulsion lipid. J Cardiol Cardiovasc Ther 5: 555660.