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Improving Longevity with Metadichol® by Inhibiting the BCAT1 Gene

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Abstract

Metadichol® [1] is a Nanoemulsion of long-chain alcohols found in many foods. It is commonly called Policosanol and is present in foods such as rice, sugar cane, wheat, peanuts Metadichol acts as an inverse agonist on Nuclear Vitamin D receptors (VDR) that are present in cells throughout the body to stimulate the immune system and affects many biologIcal processes to modulate many diseases.

Branched-chain amino acid transferase (BCAT1) catalyzes the reversible transamination of leucine, isoleucine, and valine branched-chain amino acids (BCAA) to their respective alpha-keto acids, liberating L-glutamate. When this gene is inhibited, the amino acid chains accumulated in the tissue triggering longevity in the nematodes. The health and longevity of the nematodes improved when BCAT1 was inhibited. Gabapentin has been shown to inhibit BCAT1, but IC50 is 10000 uM. Metadichol® inhibits BCAT1 with an IC50 of 3.3 um, 3000 times more potent than Gabapentin.

Keywords: Aging; BCAT1; Metadichol; VDR; Nuclear receptors; Inverse agonists; Protean agonists; Urothelial carcinomas; Ovarian cancer; Hepatocellular carcinoma; Nasopharyngeal carcinoma; Hyperglycemia

Introduction

BCAA catabolism and specifically increased activity of the corresponding enzyme, BCAT1 has been linked to various diseases (Table 1) in aging [2,3] and pathological states [4], including accelerated growth of malignant gliomas [5], decreased sepsis survival [6] and increased accumulation of liver fat [7], the latter being linked to a number of metabolic diseases [8,9]. Consistently, systemic disruption of one BCAT iso-form [10], namely BCATm, in mice increases energy expenditure and reduces body weight [11] and cancer [12,13].

Humanity has always been searching for immortality, and it has been a timeless quest. Mansfield and-and his co-workers at ETH Zurich [3] systematically researched the genomes of three different organisms and found genes present in all three were associated with the aging process. These are also present in humans. They followed the developing sequences of each organism with age and studied the manner of the expression along each stage. By comparing the amount of messenger RNA found in the cells of the animals, this allowed them to measure gene activity. Through this data, they found that the three organisms have 30 genes in common, which significantly impact the aging process.

BCAT1 related diseases	
Astrocytoma	Colitis
Glioblastoma	Crohn Disease
Glioma	Inflammatory Bowel Diseases
Malignant neoplasm of brain	Leukemia
Neoplasms, Ductal, Lobular, And Medullary	Acute leukemia
Ductal Carcinoma	Myeloid Leukemia
Carcinoma, Large Cell	Muscular Dystrophy, Duchenne
Melanoma	Muscular Dystrophy
Skin Neoplasms	Duchenne And Becker Muscular Dystrophy
Neuroendocrine Tumors	Amyotrophic Lateral Sclerosis
Nevi and Melanomas	Motor Neuron Disease
Lymphoma	Anterior Horn Cell Disease
Lymphoproliferative Disorders	TDP 43 Proteinopathies
Hematopoietic Neoplasms	Adenocarcinoma
Myeloid Leukemia, Chronic	Cystadenocarcinoma
Myeloproliferative disease	Neoplasms, Cystic, Mucinous, And Serous
Malignant neoplasm of skin	Hyperglycemia

Table 1: BCAT1 related diseases.

By blocking the mRNA to the corresponding genes only increased the lifespan by 5%. But one gene, called BCAT1 gene, when blocked increased the lifespan by nearly 25%. When this gene is blocked, the amino acid chains accumulated in the tissue triggering longevity in the nematodes.

Chang et al. [14] showed that overexpression of BCAT1 overexpression is associated with advanced tumor status, and implies adverse clinical outcomes of Urothelial carcinomas, suggesting that its role in tumor progression could serve as a prognostic biomarker and a novel therapeutic target in urothelial carcinomas.

Wang et al. [15] BCAT1 suppression led to significantly prolonged survival time in the xenograft model of advanced peritoneal epithelial ovarian cancer. And suggesting that BCAT1 is a novel therapeutic target. Work on Hepatocellular carcinoma by Xu et al. [16], who showed that BCAT1 expression was upregulated in these patients and that BCAT1 may serve as a potential molecular target for the diagnosis and treatment.

Panosyan et al. [17], showed that Glutamine, glutamate, asparagine, and aspartate are involved in an enzyme network that controls nitrogen metabolism. Branched-chain-amino-acid aminotransferase-1 and

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clinical aggressiveness of malignant gliomas were linked to augmented metabolism of amino acids.

A study by Zhou and coworkers [18] showed that gene amplification and c-Myc up-regulation are responsible for BCAT1 overexpression in primary NPC, and overexpression of BCAT1 induces cell proliferation, migration, and invasion. The results suggest that BCAT1 may be a novel molecular target for the diagnosis and treatment of nasopharyngeal carcinoma.

Given the increasing importance of BCAT1 in human diseases, Metadichol®, standard Gabapentin, and policosanol (an active ingredient in Metadichol®) a nonnano form, the same one used in the preparation of Metadichol®, was tested for its inhibitory activity using cell lines U87MG and Hs 683 cell lines. The choice of the cell lines is because they express BCAT1 and also it could be compared with the standard Gabapentin that was tested using these cell lines.

Experimental

The procedure followed is described on page 6 of European Patent application [3]. The work was outsourced and performed under by Shakti BioResearch Labs Woodbridge, CT. The experiment was duplicated.

Cell Lines and Culture Conditions: Hs683 (Catalog No. HTB-138) and U87MG (Catalog No. HTB-14) were obtained from American Type Culture Collection (ATCC). They were grown in T75 flasks in DMEM (GIBCO, Catalog No. 11965-092) and MEM (GIBCO, Catalog No. 11905-080) media, respectively, at 37°C in a humidified, 5% CO2 incubator. Both DMEM and MEM media were supplemented with 10% fetal bovine serum (ATCC, Catalog No. 30-2020) and penicillin-streptomycin (ATCC, Catalog No. 30-2300).

Proliferation Assay

Proliferation is done for 72 hours where you allow the cells for 2-3 doubling time.

Cells (2,000-3,000 cells/well) were seeded in 100 uL of specified media in a 96-well plate and incubated overnight at 37°C in a humidified, 5% CO_2 incubator. Media replaced with new 100 µL of fresh media containing various concentrations of the compounds. After 72 h incubation with the compounds at 37°C in a humidified, 5% CO_2 incubator, cell viability was measured in a luminometer after the addition of 100 µL/well Cell Titer Glo reagent (Promega). IC50s were calculated using SoftMax software.

Metadichol® (size below 60 nm) used was a 0.5% (5 mg per ml) solution in water and Vehicle was diluted 10-fold in specified media followed by 3-fold serial dilutions.

Gabapentin (Selleckchem Catalog No. S1338) (was in a phosphate buffer) was dissolved in PBS at a concentration of 0.2 M; a 10-fold dilution made in the specified media followed by 3-fold serial dilutions. Policosanol in powder form (supplied by Micro-Sphere S.A Switzerland) (the same that is used in the preparation of Metadichol®) was dissolved in DMSO (the final concentration of DMSO was 0.1%) to a concentration of 10 µM (with minimal turbidity that did not get pelleted upon centrifugation), followed by 3-fold dilutions in DMSO. 1 µL of serially diluted policosanol in DMSO was added to 500 L of media.

	Hs683	U87MG		Hs683	U87MG
	IC50 µM			IC50 µg/ml	
Metadichol	3.329	5.247	Metadichol	4.661	7.346
Gabapentin	10660	1919	Gabapentin	2214.1	3985.7
Policosanol	Inactive	Inactive	Policosanol	Inactive	Inactive

Table 2: A summary of the results for cell lines HS683 and U87MG.

Results

A summary of the results is Shown in Table 2. Data and graphs for cell lines HS683 and U87MG are shown in in Figure 1 and 2 respectively. From the Table, it is seen that Metadichol® is 3000 more potent (in µM units) than Gabapentin. Policosanol, the active ingredient of Metadichol in non-nano form, is totally inactive.

The graphs are shown in Figure 1. At higher concentrations, the % inhibition of Metadichol is over 100, and it could be due to the formulation which has a pH of 4.5 which is known to affect the cell lines used.

Discussion

Vitamin D has a role in the down-regulation of BCAT1. A likely explanation to BCAT1 inhibition lies in the work of Suzuki et al. [19] using a DNA microarray analyzed 16000 genes for changes in expression with the differentiation of human promyelocytic leukemia HL-60 cells induced by 1,25-dihydroxy D3 (Vit D3), and their work showed that BCAT1 was downregulated. Metadichol has a particle size of less than 60 nm. We have demonstrated that it binds to the vitamin D receptor (VDR) as an inverse agonist. It is the only known inverse agonist of VDR known in the medical literature, and so it is not surprising that there is inhibition of BCAT1.

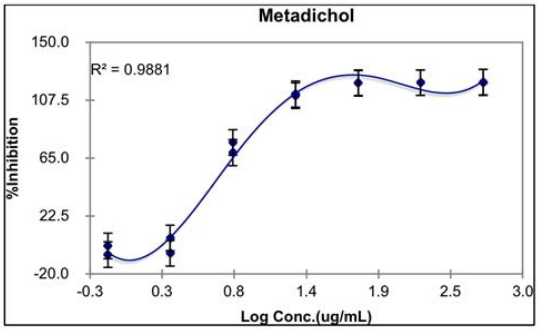
To further confirm this result we have carried out Metadichol® effect on expression and differentiation on THP-1 cell line which is a human leukemia monocyte cell line, which has been extensively used to study monocyte/macrophage functions, mechanisms, signaling pathways, and nutrient and drug transport. This cell line has become a familiar model to estimate modulation of monocyte and macrophage activities. Over 6300 genes were expressed and filtered to a set of 754 significant genes that were significant. BCAT1 is seen to be down-regulated [20].

Calcitriol (1,25-Dihydroxy Vitamin D) is the natural ligand for the VDR and acts as an agonist. Metadichol likely behaves more like a Protean agonist which act as both positive and negative agonists on the same receptor, depending on the degree of constitutive activity that is present. If there is no constitutive activity, the agonist would be an active agonist. When constitutive activity is present, the Protean agonist would be an inverse agonist [21].

Metadichol is a product made from agricultural waste and is a renewable resource. It has the potential to serve as an anti-aging molecule with a broad spectrum of activity, particularly given that its constituents (long-chain lipid alcohols are classified as GRAS). Given that they are present in foods commonly consumed on a daily basis and has demonstrated no toxicity at doses of up to 5000 mg/kg [22-24]. Given this safety record, Metadichol is ready for large scale clinical testing to prove its efficacy in BCAT1 related diseases and saving years of work in bringing a potential drug to market.

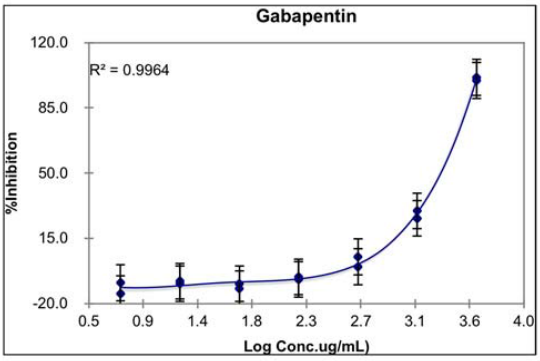
Metadichol	Log Conc	Activity
	-0.2	0.5
	-0.2	-6.0
	0.3	6.2
	0.3	-4.9
	0.8	76.4
	0.8	68.9
	1.3	111.9
	1.3	110.8
	1.7	120.1
	1.7	119.7
	2.2	120.4
	2.2	120.1
	2.7	120.6
	2.7	120.4

IC50= 4.661



Gabapentin	Log Conc	Activity
	0.8	-8.8
	0.8	-14.7
	1.2	-9.3
	1.2	-8.0
	1.7	-9.4
	1.7	-12.0
	2.2	-5.7
	2.2	-7.1
	2.7	5.1
	2.7	-0.3
	3.1	29.7
	3.1	25.6
	3.6	101.3
	3.6	99.7

IC50= 2214.1



Polycosanol	Log Conc	Activity
	-1.9	-1.5
	-1.9	6.6
	-1.5	-9.4
	-1.5	-0.3
	-1.0	-1.5
	-1.0	0.2
	-0.5	0.7
	-0.5	2.6
	-0.0	-1.4
	-0.0	-6.6
	0.4	-2.1
	0.4	8.1
	0.9	8.0
	0.9	10.2

IC50= Inactive

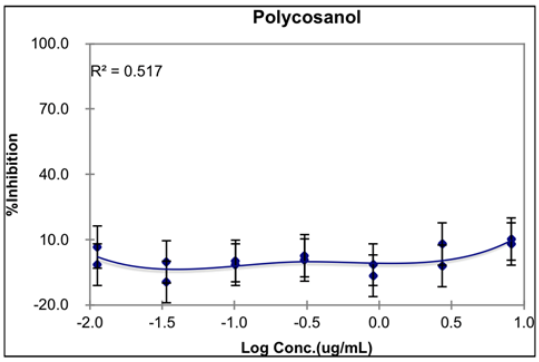
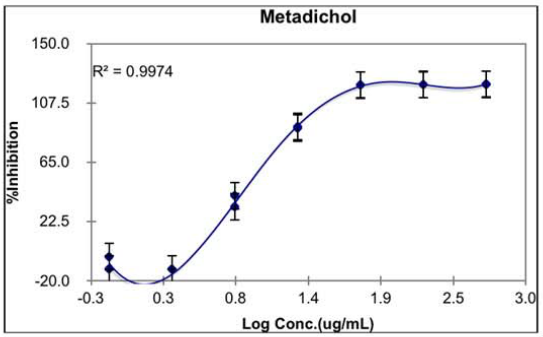


Figure 1: Data and graphs for HS 683 cell line.

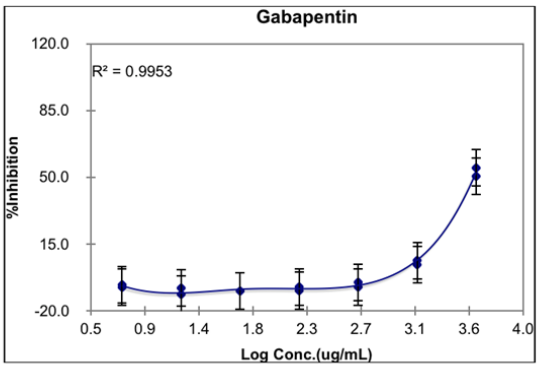
Metadichol	Log Conc	Activity
	-0.2	-2.7
	-0.2	-11.4
	0.3	-11.6
	0.3	-20.9
	0.8	41.1
	0.8	33.2
	1.3	90.5
	1.3	89.5
	1.7	120.5
	1.7	120.1
	2.2	120.8
	2.2	120.5
	2.7	121.1
	2.7	120.8

IC50= 7.346



Gabapentin	Log Conc	Activity
	0.8	-7.6
	0.8	-6.2
	1.2	-8.0
	1.2	-11.2
	1.7	-9.5
	1.7	-9.5
	2.2	-7.4
	2.2	-9.3
	2.7	-4.9
	2.7	-7.4
	3.1	4.3
	3.1	6.5
	3.6	50.6
	3.6	54.9

IC50= 3985.7



Policosanol	Log Conc	Activity
	-1.9	-1.2
	-1.9	-3.1
	-1.5	-2.2
	-1.5	-3.8
	-1.0	-2.9
	-1.0	-3.2
	-0.5	0.8
	-0.5	-2.4
	-0.0	3.3
	-0.0	0.4
	0.4	5.2
	0.4	-1.0
	0.9	1.6
	0.9	-3.0

IC50= Inactive

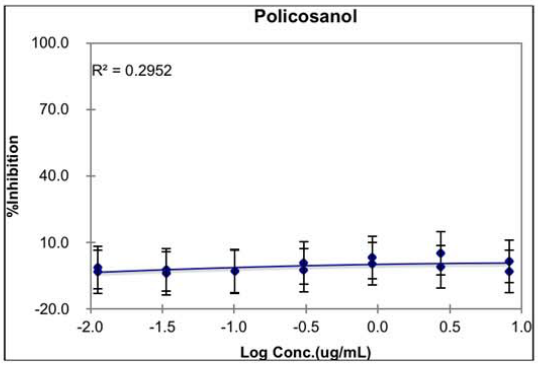


Figure 2: Data and graphs for U87 MG Cell Line.

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