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(54) Title: IDENTIFICATION OF COMPOUNDS THAT TARGET THE RNA-BINDING PROTEIN TIA-1 AN IMPORTANT REGULATOR OF STRESS VULNERABILITY IN BOTH MICE AND HUMANS

(57) **Abstract:** This invention provides a method of ameliorating the symptoms of, or treating post-traumatic stress disorder in a mammal, the method comprising administering to the mammal an effective amount of a compound that upregulates TIA-1 multimerization. This invention also provides a method of ameliorating the symptoms of, or treating post-traumatic stress disorder in a mammal, the method comprising administering to the mammal an effective amount of a compound that inhibits TIA-1 multimerization. This invention also provides a method for determining whether a predetermined compound upregulates or inhibits TIA-1 multimerization, using fluoresence resonance energy transfer (FRET), the method comprising: expressing recombinant TIA-CFP and TIA-YFP; exposing the recombinant TIA-CFP and TIA-YFP to such predetermined compound; exciting the TIA-CFP with a laser; and measuring the fluorescence of TIA-YFP, wherein an increase in fluorescence relative to TIA-CFP and TIA-YFP not exposed to the predetermined compound indicates that the predetermined compound upregulates TIA-1 multimerization and a decrease in fluorescence relative to TIA-CFP and TIA-YFP not exposed to the predetermined compound indicates that the predetermined compound inhibits TIA-1 multimerization.

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IDENTIFICATION OF COMPOUNDS THAT TARGET THE RNA-BINDING PROTEIN TIA-1, AN IMPORTANT REGULATOR OF STRESS VULNERABILITY IN BOTH MICE AND HUMANS

This application claims priority of U.S. Provisional Patent Application No. 62/351,787, filed June 17, 2016, the entire contents of which are hereby incorporated herein by reference.

Throughout this application, various publications are referenced by author and publication date within parentheses. Full citations for these publications may be found at the end of the specification or at the end of each experimental section. The disclosures of these publications are hereby incorporated by reference into this application to describe more fully the art to which this invention pertains.

Background of the Invention

Post-traumatic stress disorder (PTSD) is a mental disorder that develops after an individual experiences a traumatic disorder such as sexual assault, abuse, death, or warfare. However, the majority of people who experience a traumatic event will not develop PTSD. Factors that contribute one's susceptibility to PTSD are unclear. Additionally, psychotherapy is the best treatment; pharmacological agents have been largely ineffective.

Summary of the Invention

This invention provides a method of ameliorating the symptoms of, or treating post-traumatic stress disorder in a mammal, the method comprising administering to the mammal an effective amount of a compound that upregulates TIA-1 multimerization.

This invention also provides a method of ameliorating the symptoms of, or treating post-traumatic stress disorder in a mammal, the method comprising administering to the mammal an effective amount of a compound that inhibits TIA-1 multimerization.

This invention also provides a method for determining whether a predetermined compound upregulates or inhibits TIA-1 multimerization, using fluoresence resonance energy transfer (FRET), the method comprising:

a. expressing recombinant TIA-CFP and TIA-YFP;

b. exposing the recombinant TIA-CFP and TIA-YFP to such predetermined compound;

c. exciting the TIA-CFP with a laser; and

d. measuring the fluorescence of TIA-YFP,

wherein an increase in fluorescence relative to TIA-CFP and TIA-YFP not exposed to the predetermined compound indicates that the predetermined compound upregulates TIA-1 multimerization and a decrease in fluorescence relative to TIA-CFP and TIA-YFP not exposed to the predetermined compound indicates that the predetermined compound inhibits TIA-1 multimerization.

Detailed Description of the Invention

Embodiments of the Invention

This invention provides a method of ameliorating the symptoms of, or treating post-traumatic stress disorder in a mammal, the method comprising administering to the mammal an effective amount of a compound that upregulates TIA-1 multimerization.

In one embodiment, compound is D-alpha- tocopherol succinate (TS) or a pharmaceutically acceptable salt thereof.

This invention also provides a method of ameliorating the symptoms of, or treating post-traumatic stress disorder in a mammal, the method comprising administering to the mammal an effective amount of a compound that inhibits TIA-1 multimerization.

In one embodiment the compound is oxytetracyline dihydrate or a pharmaceutically acceptable salt thereof.

In one embodiment the compound is moxalactam disodium or a pharmaceutically acceptable salt thereof.

In one embodiment the compound is L-ascorbate or a pharmaceutically acceptable salt thereof.

This invention also provides a method for determining whether a predetermined compound upregulates or inhibits TIA-1 multimerization, using fluoresence resonance energy transfer (FRET), the method comprising:

a. expressing recombinant TIA-CFP and TIA-YFP;

b. exposing the recombinant TIA-CFP and TIA-YFP to such predetermined compound;

c. exciting the TIA-CFP with a laser; and

d. measuring the fluorescence of TIA-YFP,

wherein an increase in fluorescence relative to TIA-CFP and TIA-YFP not exposed to the predetermined compound indicates that the predetermined compound upregulates TIA-1 multimerization and a decrease in fluorescence relative to TIA-CFP and TIA-YFP not exposed to the predetermined compound indicates that the predetermined compound inhibits TIA-1 multimerization.

Examples

TIA-1 is a prion-related RNA binding protein that regulates multiple aspects of RNA metabolism, including translation and alternative splicing. Unpublished work by the inventors has shown that TIA-1 knockout (KO) mice are more vulnerable to the effects of traumatic stress (fear conditioning) than wild-type littermates. For example, one of the key symptom clusters of post-traumatic stress disorder (PTSD) is avoidance behavior, which is enhanced in KO mice several weeks after exposure to fear conditioning. Moreover, this and other stress-induced behavioral phenotypes occur only in female KO mice, and are accompanied by altered synaptic plasticity in areas of the brain that are critical for the normal processing of fear memory, such as the hippocampus and amygdala. In turn, these behavioral and electrophysiological phenotypes are driven by TIA-dependent changes in alternative splicing of the glucocorticoid receptor, a critical regulator of the adaptive stress response. Based on these findings polymorphisms in the human TIA-1 gene that interact with exposure to stressful life events have been identified which predict the development of pathological anxiety in people. Taken together, these data indicate that TIA-1 plays a critical role in the behavioral response to stress in both mice and humans. Furthermore, these findings suggest that TIA-1 may be a useful therapeutic target in the treatment of stress-related psychiatric disorders such as PTSD.

The inventors hypothesized that physiological aggregation of TIA-1 is essential for dynamic splicing of the glucocorticoid receptor during stress, and that aggregation serves a positive function in this context. By extension, the inventors propose that TIA aggregation may be therapeutically modulated by pharmacological intervention.

Identification of compounds that target TIA-1

Prions are classically regarded as agents of neurodegenerative disease, where aggregation ultimately leads to cell death. However, there is evidence that a number of prion-related proteins such as TIA-1 serve normal physiological roles in their aggregated state. Thus, the functional state of TIA-1 is likely determined by whether it exists in monomeric versus aggregated conformation. To explore the function of TIA-1 aggregation, we established a novel FRET-based assay to study the interaction between recombinant TIA-1 proteins in vitro (TIA-CFP and

TIA-YFP, a compatible FRET pair). In this assay, laser excitation of CFP leads to emission at a characteristic range of wavelengths which, in turn, can excite YFP only if the two recombinant proteins are essentially bound to one another. Given that TIA-1 is highly responsive to redox changes in the cell, the inventors began screening an antioxidant compound library, observing the effects of each drug on TIA-1 FRET signal and therefore TIA-1 homotypic interaction (multimerization or aggregation). In this manner, the inventors identified at least one compound that upregulates TIA- 1 self-interaction (D-alpha-tocopherol succinate), and at least one drug that inhibits interaction (L-ascorbate). Furthermore, D-alpha-tocopherol succinate (TS) administration (a single i.p. injection at 10 mg/kg) led to the formation of massive, high molecular weight complexes containing TIA-1 in the mouse hippocampus within 30 min. of injection, as observed by SDS-agarose gel analysis. Importantly, these types of aggregates are also observed for endogenous TIA-1 in fear conditioned mice within 30' of training, which implies the physiological relevance of this structural state.

Inventors have also identified two additional compounds that inhibit TIA-1 multimerization in vitro: oxytetracyline dihydrate and moxalactam disodium.

Animal studies

These results prompted inventors to test the effects of candidate compounds on stress-induced behavior. Inventors first established baseline performance of naïve wild-type mice in the conditioned odor paradigm. Twenty-four hours later, mice were trained in contextual fear conditioning (standard protocol of 2 x 2 sec. shocks at .7 mA), and then either TS, L-ascorbate (LA), or vehicle (DMSO) were injected (i.p., 10 mg/kg) one hour later. Approximately 3 weeks later, mice were evaluated once again in the odor avoidance paradigm, among other behavioral measures, and then tested for long-term explicit fear memory the next day. In this first animal study, inventors found that TS increases avoidance behavior rather than reduces it, contrary to expectations. Intriguingly, this effect appears to be specific to female mice. However, contextual fear memory is also strengthened in TS-treated mice, independent of sex. Inventors speculate that other drug administration regimes may lead to other, more predictable

outcomes. In contrast, LA does not affect explicit fear memory, but does exacerbate avoidance behavior, as expected for a drug that blocks aggregation of TIA-1.

Further Animal Studies

As mentioned above, a FRET-based drug screen using purified, recombinant TIA-1 led to the identification of D-alpha-tocopherol succinate as a potent inducer of TIA-1 multimerization. Other tocopherol isoforms—alpha-tocopherol, tocopherol acetate, and gammatocopherol—have no effect in vitro. Furthermore, injecting tocopherol succinate into naive mice causes endogenous TIA-1 to form high molecular weight, SDS-resistant complexes that may be comprised of TIA-1 multimers. Finally, a single intraperitoneal injection of tocopherol succinate (10 mg/kg) into mice within 1 hour after fear conditioning significantly enhances avoidance behavior at 3 weeks following training. As discussed earlier, this result is essentially the opposite of what we would predict for this particular manipulation.

However, in further testing, inventors subsequently determined that a lower dose of the compound (4 mg/kg) reduces avoidance behavior in these animals (i.e., ameliorates PTSD-like symptoms). These observations suggest that the effect of TIA-1 aggregation on fear memory processing is governed by an "inverted-U" function, whereby too much or too little aggregation is maladaptive for the organism.

Animal studies are underway to evaluate the effects of oxytetracyline dihydrate and moxalactam disodium on fear and memory related behavioral phenotypes.

Discussion

There are currently only 2 FDA-approved drugs for the treatment of PTSD. Novel and effective therapeutics are badly needed.

While initial animal studies have not produced completely anticipated behavioral outcomes (i.e., reduction of PTSD-like symptoms by TS treatment), these studies are at a very early stage, and it is very clear that inventors have succeeded in targeting an important stress response pathway that modulates how fear memory is processed. Other drug treatment regimes remain to be evaluated, and may modulate this fear response pathway in more desirable ways.

As stated above, the results of initial experiments on mice suggest that the effect of TIA-1 aggregation on fear memory processing is governed by an "inverted-U" function, whereby too much or too little aggregation is maladaptive for organism.

Using the inventors FRET assay, described herein, it is possible to screen for compounds that increase or decrease TIA-1 multimerization. As shown herein, such compounds are potentially useful for treating PTSD or PTSD-related symptoms. Further, the same mice behavioral studies, described herein, can be used to determine the therapeutic effects of any of various dosage amounts any other compound identified as a modulator of TIA-1 multimerization (such as oxytetracyline dehydrate, moxalactam disodium, L-ascorbate, or others identified in the future as affecting TIA-1 multimerization).

The U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) provides a table of Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area (Table 1). Data from mice studies can therefore be converted to approximate therapeutically effective doses in humans.

Table 1: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area

Species	To Convert Animal Dose in mg/kg to Dose in mg/m^2, Multiply by k_m	To Convert Animal Dose in mg/kg to HED[a] in mg/kg, Either:	
		Divide Animal Dose By	Multiply Animal Dose By
Human	37	---	---
Child (20 kg)[b]	25	---	---
Mouse	3	12.3	0.08
Hamster	5	7.4	0.13
Rat	6	6.2	0.16
Ferret	7	5.3	0.19
Guinea pig	8	4.6	0.22
Rabbit	12	3.1	0.32
Dog	20	1.8	0.54
Primates:			
Monkeys[c]	12	3.1	0.32
Marmoset	6	6.2	0.16
Squirrel monkey	7	5.3	0.19
Baboon	20	1.8	0.54
Micro-pig	27	1.4	0.73
Mini-pig	35	1.1	0.95

[a] Assumes 60 kg human. For species not listed or for weights outside the standard ranges, HED can be calculated from the following formula:

HED = animal dose in mg/kg x (animal weight in kg/human weight in kg)$^{0.33}$.

[b] This k_m value is provided for reference only since healthy children will rarely be volunteers for phase 1 trials.

[c] For example, cynomolgus, rhesus, and stumptail.

As an example the 4 mg/kg dosage of TS that was determined to ameliorate PTSD-like symptoms in mice would be approximately equivalent to 0.325 mg/kg in humans.

The search for novel therapies is of paramount importance. The drugs (and target) identified in the study represent a completely novel biochemical pathway that may be targeted to modulate adaptive behavior and long-term memory in the context of traumatic stress. Also, while glucocorticoid treatment has shown some potential in treating PTSD, its application is limited because of toxic side effects. The drugs identified in this study target the glucocorticoid pathway, but have little or no known toxicity.

What is claimed is:

1. A method of ameliorating the symptoms of, or treating post-traumatic stress disorder in a mammal, the method comprising administering to the mammal a therapeutically effective amount of a compound that upregulates TIA-1 multimerization.

2. The method of claim 1 wherein the compound is D-alpha- tocopherol succinate (TS) or a pharmaceutically acceptable salt thereof.

3. The method of claim 2, wherein the mammal is a human and the therapeutically effective amount is around 0.325 mg/kg.

4. A method of ameliorating the symptoms of, or treating post-traumatic stress disorder in a mammal, the method comprising administering to the mammal a therapeutically effective amount of a compound that inhibits TIA-1 multimerization.

5. The method of claim 3 wherein the compound is oxytetracyline dihydrate or a pharmaceutically acceptable salt thereof.

6. The method of claim 3 wherein the compound is moxalactam disodium or a pharmaceutically acceptable salt thereof.

7. The method of claim 3 wherein the compound is L-ascorbate or a pharmaceutically acceptable salt thereof.

8. A method for determining whether a predetermined compound upregulates or inhibits TIA-1 multimerization, using fluoresence resonance energy transfer (FRET), the method comprising:

   a. expressing recombinant TIA-CFP and TIA-YFP;

   b. exposing the recombinant TIA-CFP and TIA-YFP to such predetermined compound;

   c. exciting the TIA-CFP with a laser; and

   d. measuring the fluorescence of TIA-YFP,

   wherein an increase in fluorescence relative to TIA-CFP and TIA-YFP not exposed to the predetermined compound indicates that the predetermined compound upregulates TIA-1 multimerization and a decrease in fluorescence relative to TIA-CFP and TIA-YFP not exposed to the predetermined compound indicates that the predetermined compound inhibits TIA-1 multimerization.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/037544

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/17; C07K 14/435; C07K 14/47; G01N 33/68 (2017.01)

CPC - A61K 38/1761; C07K 14/4747; C07K 2319/60; G01N 33/6896; G01N 2500/02 (2017.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 436/501; 514/17.7; 514/18.9; 702/19 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2015/0139972 A1 (PREMIER MICRONUTRIENT CORP et al) 21 May 2015 (21.05.2015) entire document	1-3
Y	ARIMOTO-MATSUZAKI et al. "TIA1 oxidation inhibits stress granule assembly and sensitizes cells to stress-induced apoptosis," Nature Communications, 07 January 2016 (07.01.2016), Vol. 7:10252, Pgs. 1-10. entire document	1-3
Y	US 2012/0034193 A1 (REES et al) 09 February 2012 (09.02.2012) entire document	3
Y	US 9,309,196 B2 (ABEL) 12 April 2016 (12.04.2016) entire document	4-7
Y	ASH et al. "Pathological Stress Granules in Alzheimer's Disease," Brain Research, 07 August 2014 (07.08.2014), Vol. 1584, Pgs. 52-58. entire document	4-7
Y	US 2004/0014739 A1 (KOPPEL) 22 January 2004 (22.01.2004) entire document	6
Y	WO 2015/160843 A1 (FLEX PHARMA, INC. et al) 22 October 2015 (22.10.2015) entire document	7
Y	US 7,157,566 B2 (TSIEN et al) 02 January 2007 (02.01.2007) entire document	8
Y	LI et al. "Functional Role of Tia1/Pub1 and Sup35 Prion Domains: Directing Protein Synthesis Machinery to the Tubulin Cytoskeleton," Molecular Cell, 17 July 2014 (17.07.2014), Vol. 55, No. 2, Pgs. 305-318. entire document	8

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/037544

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JUSTICE et al. "Posttraumatic Stress Disorder-Like Induction Elevates β-Amyloid Levels, Which Directly Activates Corticotropin-Releasing Factor Neurons to Exacerbate Stress Responses," Journal of Neuroscience, 11 February 2015 (11.02.2015), Vol. 35, No. 6, Pgs. 2612-2623. entire document	1-8
A	PRUSINER. "Biology and Genetics of Prions Causing Neurodegeneration," Annual Review of Genetics, 05 May 2014 (05.05.2014), Vol. 47, Pgs. 601-623. entire document	1-8
A	WO 2015/025323 A1 (YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM LTD.) 26 February 2015 (26.02.2015) entire document	1-8