APPLICATION OF THYROID HORMONES AND ITS ANALOGUES IN PREPARATION OF DRUGS FOR TREATING ALPHA-THALASSEMIA

BACKGROUND OF THE INVENTION

[0001] 1. Technical Field

[0002] The present invention belongs to the technical field of thalassemia, and particularly relates to an application of thyroid hormones and its analogues in preparation of drugs for treating alpha-thalassemia.

[0003] 2. Description of Related Art

[0004] The thalassemias are the most common monogenic diseases in the world, which have great influences on human health. These inherited disorders of hemoglobin synthesis are characterized by a reduced production of globin chains of hemoglobin. Worldwide, the most important forms are the alpha- and beta-thalassemias, which affect production of the alpha-globin and beta-globin chains, respectively. The number of alpha-thalassemia gene carriers is higher than that of beta-thalassemia gene carriers, and the number of patients with alpha-thalassemia intermediate (also known as HbH disease) and alpha-thalassemia major (also known as Hemoglobin Bart's hydrops fetalis) in China is much higher than that in other countries and regions of the world, so China is a veritable "hardest hit area" of alpha-thalassemia. However, there is no mature and effective method for treating such diseases so far. Therefore, patients with alpha-thalassemia face huge unmet medical demands.

[0005] Alpha-thalassemia refers to a hemolytic anemia caused by the destruction of erythrocytes due to a decrease of the synthesis of alpha-globin chains. There are four types of alpha-thalassemia, which are as follows: (1) silent alpha-carriers; (2) alpha-thalassemia minor; (3) alpha-thalassemia intermediate (also known as HbH disease); and (4) alpha-thalassemia major (also known as Hemoglobin Bart's hydrops fetalis). The patients with silent alpha-carriers and alpha-thalassemia minor do not need special treatment. However, some patients with HbH disease need blood transfusion. For infants with Hemoglobin Bart's hydrops fetalis, some hospitals have reported that intrauterine blood transfusion can relieve their symptoms, but up to now, there is still no effective treatment.

[0006] However, blood transfusion treatment may bring the following problems:

[0007] 1. Blood transfusion will lead to iron overload to damage multiple organs, mainly affecting heart, liver, pancreas and various endocrine organs;

[0008] 2. Blood transfusion will cause fever, chills, rash and other adverse effects, and will lead to acute hemolysis, tracheal contraction and blood pressure drop in severe cases;

[0009] 3. Blood transfusion may pose a risk of infectious diseases through blood;

[0010] 4. Intrauterine blood transfusion has high requirements and difficulty in technique, which makes it difficult to be widely used.

[0011] 5. Blood transfusion has high cost, which will cause an economic burden especially for patients who need lifelong blood transfusion.

[0012] Zeta-globin is a type of alpha-like globin expressed only in an embryonic stage, which also has an oxygen-carrying function. A gene encoding this protein is well preserved in most patients with alpha-thalassemia, and its expression is gradually silenced during embryonic development. Studies have proved that in a lethal alpha-thalassemia mouse model, re-expression of zeta-globin by a transgenic method can fully restore normal development of the alpha-thalassemia mouse. Therefore, reactivation of zeta-globin gene expression in patients to replace the defective alpha-globin is expected to be an effective method for treating alpha-thalassemia. However, pharmacologic compounds capable of reactivating zeta-globin gene expression have not been yet available so far.

BRIEF SUMMARY OF THE INVENTION

[0013] To overcome the shortcomings of the prior art, the primary purpose of the present invention is to provide an application of thyroid hormones and its analogues in preparation of drugs for treating alpha-thalassemia.

[0014] A second purpose of the present invention is to provide an application of thyroid hormones and its analogues in preparation of drugs for regulating expression of zeta-globin gene.

[0015] To achieve the above primary purpose, the present invention provides the following solution:

[0016] Thyroid hormones and its analogues can be applied to preparation of drugs for treating alpha-thalassemia.

[0017] To achieve the above second purpose, the present invention provides the following solution:

[0018] Thyroid hormones and its analogues can be applied to preparation of drugs for regulating expression of zeta-globin gene.

[0019] Specifically, the thyroid hormones and its analogues can significantly up-regulate the expression of zeta-globin gene.

[0020] Triac (also known as Tiratricol or 3,3',5-triiodothyroacetic acid) (chemical name: 2-[4-(4-hydroxy-3-iodophenoxy)-3,5-diiodophenyl] acetic acid; molecular formula: $C_{14}H_9I_3O_4$) is a thyroid hormone analogue for treating patients with thyroid hormone resistance syndrome, hyperlipidemia, localized lipodystrophy and obesity. In addition, Triac has also displayed therapeutic potential for the treatment of Allan-Herndon-Dudley syndrome.

[0021] The inventor has found that during the differentiation of K562 cells, Triac can significantly up-regulate the expression of zeta-globin gene (*HBZ*). Furthermore, Triac could also dramatically induce zeta-globin gene (*hbae5*) expression in zebrafish. In addition, the inventor has also found that the thyroid hormones (3,3',5-Triiodo-L-thyronine, referred to as T3 or 3,3',5,5'-tetraiodothyronine, referred to as T4) had a similar effect. Therefore, the inventor discovered for the first time that the thyroid hormone analogue (Triac) and thyroid hormones (T3 or T4) could significantly induce zeta-globin gene expression both in vitro and in vivo, which may allow development of new therapies for alpha-thalassemia.

[0022] Specifically, Triac was purchased from Selleck Biotech Co., Ltd. of the United States, T3 (3,3',5-Triiodo-L-thyronine) was purchased from Sigma Company, and T4 (3,3',5,5'-tetraiodothyronine) was purchased from Biological Engineering (Shanghai) Ltd., Co.

[0023] By adopting the above solutions, the present invention has the following beneficial effects:

[0024] according to the present invention, thyroid hormones (T3 and T4) and thyroid hormone analogue (Triac) act as a potent inducer of zeta-globin expression, which may sever as a new potential therapeutic option for patients with alpha-thalassemia. It provides an economical, safe and effective method for the treatment of alpha-thalassemia, and can be widely used.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0025] Fig. 1. The thyroid hormone analogue (Triac) induced zeta-globin gene (*HBZ*) expression in hemin-treated K562 cells.

[0026] Fig. 2. WISH assay shows Triac, T3 or T4 induced zeta-globin gene (*hbae5*) expression in zebrafish embryos.

[0027] Fig. 3. qPCR shows Triac, T3 or T4 induced zeta-globin gene (*hbae5*) expression in zebrafish embryos.

DETAILED DESCRIPTION OF THE INVENTION

[0028] The present invention provides an application of thyroid hormones and its analogues in preparation of drugs for treating alpha-thalassemia.

[0029] Experimental materials:

[0030] K562 cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum; Hemin was dissolved into 0.2 mol/L NaOH solution, and the working concentration for treating K562 cells is 20 μmol/L; Triac, T3 and T4 were dissolved into dimethyl sulfoxide (DMSO), and the working concentration for treating K562 cells and zebrafish embryos is 20 μmol/L and 5 μmol/L, respectively.

[0031] The present invention is further described below through specific embodiments.

[0032] Embodiment 1:

[0033] In K562 cells, Triac significantly activated the expression of zeta-globin gene (*HBZ*) according to the following process:

[0034] K562 cells were treated with hemin or hemin plus Triac for 72 hours, respectively, centrifugation was performed at 2000 rpm for 5 minutes to collect cells, a TRIzol reagent (Invitrogen) was used to extract total RNA, then a reverse transcription kit (ReverTra Ace, TOYOBO) was used to synthesize cDNA, and finally, a SYBR Green Realtime PCR Master Mix (TOYOBO) reagent was used to perform quantitative PCR.

[0035] A quantitative PCR reaction system was as follows:

[0036]	2xSYBR Green PCR Master Mix	5 ul
[0037]	F-Primer (10 μmol/L)	0.6 ul
[0038]	R-Primer (10 μmol/L)	0.6 ul
[0039]	cDNA	1 ul
[0040]	ddH2O	2.8 ul
[0041]	Total volume	10ul

[0042] Conditions for a quantitative PCR reaction were as follows:

[0043] Hot start: 95°C, 10 minutes;

[0044] Denaturation: 95°C, 10 seconds; and

[0045] Annealing/stretching: 60°C, 30 seconds.

[0046] 40 cycles.

[0047] Dissolution curve analysis.

[0048] As shown in Fig. 1, qPCR result shows that the expression of zeta-globin gene (*HBZ*) is up-regulated by 50.9 folds in Triac-treated K562 cells.

[0049] In fact, the sequence of human zeta-globin gene (*HBZ*) (NM_005332.3) (SEQ ID NO.1) is as follows:

[0050] ATGTCTCTGACCAAGACTGAGAGGACCATCATTGTGTCCATGTGG GCCAAGATCTCCACG

[0051] CAGGCCGACACCATCGGCACCGAGACTCTGGAGAGGCTCTTCCT CAGCCACCCGCAGACC

[0052] AAGACCTACTTCCCGCACTTCGACCTGCACCCGGGGTCCGCGCA GTTGCGCGCGCACGGC

[0053] TCCAAGGTGGTGGCCGCCGTGGGCGACGCGGTGAAGAGCATCG ACGACATCGGCGGCGCC

[0054] CTGTCCAAGCTGAGCGAGCTGCACGCCTACATCCTGCGCGTGGA CCCGGTCAACTTCAAG

[0055] CTCCTGTCCCACTGCCTGCTGGTCACCCTGGCCGCGCGCTTCCCC GCCGACTTCACGGCC

[0056] GAGGCCCACGCCGCCTGGGACAAGTTCCTATCGGTCGTATCCTCT GTCCTGACCGAGAAG

[0057] TACCGCTGA.

[0058] Realtime PCR primers are as follows:

[0059] Human-β-actin-F CCAACCGCGAGAAGATGA (SEQ ID NO.2)

[0060] Human-β-actin-R CCAGAGGCGTACAGGGATAG (SEQ ID NO.3)

[0061] Human-HBA1/2-F AAGGTCGGCGCGCACGC (SEQ ID NO.4)

[0062] Human-HBA1/2-R CTCAGGTCGAAGTGCGGG (SEQ ID NO.5)

[0063] Human-HBZ-F GGACCATCATTGTGTCCATGT (SEQ ID NO.6)

[0064] Human-HBZ-R GGGAAGTAGGTCTTGGTCTGC (SEQ ID NO.7)

[0065] Human-HBE1-F TGCATGTGGATCCTGAGAAC (SEQ ID NO.8)

[0066] Human-HBE1-R CGACAGCAGACACCAGCTT (SEQ ID NO.9)

[0067] Human-HBG1/2-F AGCACCTGGATGATCTCAAG (SEQ ID NO.10)

[0068] Human-HBG1/2-R AAACGGTCACCAGCACATTT (SEQ ID NO.11)

[0069] Human-HBD-F GATGCAGTTGGTGGTGAGG (SEQ ID NO.12)

[0070] Human-HBD-R GGGTTGCCCATAACAGCAT (SEQ ID NO.13)

[0071] Human-HBB-F GCACGTGGATCCTGAGAACT (SEQ ID NO.14)

[0072] Human-HBB-R CACTGGTGGGGTGAATTCTT (SEQ ID NO.15)

[0073] Embodiment 2:

[0074] During the development of zebrafish embryos, Triac, T3 and T4 were incubated with the zebrafish embryos at 96 hours post fertilization. After treatment with Triac, T3 and T4 for 24 hours, respectively, zebrafish embryos were fixed by 4% paraformaldehyde. The expression of zeta-globin gene (*hbae5*) was detected by Whole-mount mRNA in situ hybridization (WISH).

[0075] As shown in Fig. 2, Triac, T3 and T4 significantly induce the expression of zeta-globin gene (*hbae5*) in zebrafish embryos.

[0076] Embodiment 3:

[0077] During the development of zebrafish embryos, Triac, T3 and T4 were incubated with the zebrafish embryos at 96 hours post fertilization. After treatment with Triac, T3 and T4 for 24 hours, respectively, zebrafish embryos were collected. TRIzol reagent (Invitrogen) was used to extract total RNA, then a reverse transcription kit (ReverTra Ace, TOYOBO) was used to synthesize cDNA, and finally, a SYBRGreen Realtime PCR Master Mix（TOYOBO）reagent was used to perform quantitative PCR.

[0078] A quantitative PCR reaction system was as follows:

[0079] 2xSYBR Green PCR Master Mix	5 ul
[0080] F-Primer (10 μmol/L)	0.6ul
[0081] R-Primer (10 μmol/L)	0.6ul
[0082] cDNA	1 ul
[0083] ddH2O	2.8 ul
[0084] Total volume	10 ul

[0085] Conditions for a quantitative PCR reaction were as follows:

[0086] Hot start: 95°C, 10 minutes;

[0087] Denaturation: 95°C, 10 seconds; and

[0088] Annealing/stretching: 60°C, 30 seconds.

[0089] 40 cycles.

[0090] Dissolution curve analysis.

[0091] As shown in Fig. 3, qPCR result shows that the expression of zeta-globin gene (*hbae5*) is up-regulated by 76-folds, 31-folds and 41-folds in Triac, T3 and T4-treated zebrafish embryos, respectively.

[0092] In fact, the sequence of zebrafish zeta-globin gene (*hbae5*) (NM_001326701.1) (SEQ ID NO.16) is as follows:

[0093] ATGAGTCTTTCTGCTAAAGACAAGGCCGCCGTGAGGGGCTTCTG GGCCAAGATTGCCCCA

[0094] AAGGGAGAGCAAATTGGTAACGAGGCGTTTTCCAGATTGCTTTT GGTGTACCCTCAGACC

[0095] AAGACCTACTTCTCCCACTGGAACGATCTGGCCCCCGGCTCTCCC TCTGTGAAGAAGCAG

[0096] GGAAAGAAGATCGTCGGTGGACTCGGTCTGGCTGTTGATAAAAT CGACGACCTTTTCAAC

[0097] GGCCTGCTGAACCTCAGTGAATTGCACGCCTTTCAGCTGAGAGT CGACCCTGCTAACTTC

[0098] AAGCTCCTGTCTCACTGTCTGCTGGTGGTGTTCGCCATGCTCTTC CCTGATGACTTCACC

[0099] GCTGAGGTCCATCTGGCCATCGACAAGTTCCTGGCAAGAGTGGC TTTGGCTCTGTCTGAC

[00100] AAATATCGTTAA.

[00101] The Realtime PCR primers are as follows:

[00102] Zebrafish-β-actin-F TGCTGTTTTCCCCTCCATTG (SEQ ID NO.17)

[00103] Zebrafish-β-actin-R TTCTGTCCCATGCCAACCA (SEQ ID NO.18)

[00104] Zebrafish-hbae1-F CTGAGGCTGTCAGCAAAATCG (SEQ ID NO.19)

[00105] Zebrafish-hbae1-R GAACAAAGTGGCCAGAACCAC (SEQ ID NO.20)

[00106] Zebrafish-hbae3-F GCTGATGGATGACCTGAAGGG (SEQ ID NO.21)

[00107] Zebrafish-hbae3-R CTCAGGAGTGAAGTCGTCTGG (SEQ ID NO.22)

[00108] Zebrafish-hbae5-F TGCTGAACCTCAGTGAATTGC (SEQ ID NO.23)

[00109] Zebrafish-hbae5-R GGAACTTGTCGATGGCCAGAT (SEQ ID NO.24)

[00110] Zebrafish-hbbe1-F TCCACGTAGATCCCGACAAC (SEQ ID NO.25)

[00111] Zebrafish-hbbe1-R TACTGTCTTCCCAGAGCGGA (SEQ ID NO.26)

[00112] Zebrafish-hbbe2-F GGACTGGACAGAGCCATGAAG (SEQ ID NO.27)

[00113] Zebrafish-hbbe2-R GAGGCAATCACGATTGTCAGG (SEQ ID NO.28)

[00114] Zebrafish-hbbe3-F TTGTGTGGACAGCTGAGGAG (SEQ ID NO.29)

[00115] Zebrafish-hbbe3-R ACGGATAGACGACCAAGCAT (SEQ ID NO.30)

[00116] The above description of the embodiments is intended to facilitate the understanding and use of the present invention by those of ordinary skill in the art. It will be apparent for those skilled in the art to make various modifications to these embodiments and to apply the general principle described herein to other embodiments

without creative effort. Therefore, the present invention is not limited to the above embodiments. Improvements and modifications made by those skilled in the art in accordance with the principle of the present invention without departing from the scope of the present invention should fall within the scope of the present invention.

What is claimed is:

1. Use of Triac, a thyroid hormone analogue, in the manufacture of a medicament for treating alpha-thalassemia.

2. A thyroid hormone analogues according to claim 1, wherein Triac up-regulates expression of zeta-globin gene.