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Article

Intraperitoneal injection adenovirus pmultirnai-ldhc okay plateau pika (*ochotona curzoniae*) skeletal muscle ldhc gene of silence effect

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Abstract: Intraperitoneal injection is a kind of simple and convenient to drug of style in order to verify intraperitoneal injection adenovirus Pmultirnai-ldhc. The plateau pika (*Ochotona curzoniae*) Skeletal Muscle Ldhc Gene Silence of feasibility will 27 Only plateau pika (Ochotona curzoniae) divided into interference group, Shell Group and blank control group each group9Only individual interference group and Shell Group respectively Injection0.65 mL Adenovirus Pmultirnai-ldhc And adenovirus Pmultirnai-NSBlank control group injected with the same amount of physiological saline injection after7/d Detection Skeletal Muscle in Ldhc Gene MRNA And protein of Expression Level Determination the lactate dehydrogenase (LDH) Activity and lactic acid (LD) And three phosphoric acid adenosine (ATP) Of content. Results show that and blank control group compared interference group in MRNA AND PROTEIN LEVEL, Ldhc Gene expression respectively reduce 41.73% And 15.76%; Lactate dehydrogenase (LDH) Activity, lactic acid (LD) And ATP Content respectively reduce 23.98%,51.08%And19.29%. Results (intraperitoneal injection adenovirus Pmultirnai-ldhcCan effective silence Skeletal Muscle in Ldhc Gene expression.

Keywords: intraperitoneal injection; plateau pika; lactate dehydrogenase; lactic acid; three phosphoric acid adenosine

Intraperitoneal injection is a common method of administration, which is easy to operate and easy to control; Duan (2016) Rats were built by injecting cyclophosphamide (Rattus norvegicus) The model found that the metabolites of small molecules absorbed by the peritoneal cavity acted on the whole body. After intraperitoneal injection of macromolecular substances, they are absorbed by capillaries and lymphatic vessels in the superior mesenteric region, and then enter the liver through the portal vein. After the first pass effect of the liver, the metabolism becomes non-toxic or low toxic substances and then enters the body circulation, reduce adverse reactions in animals [1], Which also causes drug depletion and reduces the amount of drugs entering the blood. Macromolecular adenovirus Pmultirnai-ldhc After intraperitoneal injection, the liver can effectively reach the skeletal muscle and play a role.7 d Effects of Adenovirus on Skeletal Muscle of plateau pika Ldhc Gene silencing effect.

1. Materials and methods

1.1. Experimental animals

Plateau pika captured in the ridge mountain of guide County, Hainan State, Qinghai Province, sample size 27 Only, weight150~200 g, randomly divided 3 Groups, per group 9 only. No. 1 Group as interference group (RNAi-ldhc),

Intraperitoneal injection $4x10^9$·PFU (The number of virus particles) 0.65 Adenovirus Pmultirnai-ldhc. 2 Groups are empty groups (RNAi-HK), injecting the same dose of adenovirus Pmultirnai-NS No. 3 Control group (Control), injecting the same dose of saline. Plateau pika kept after injection 7d, Resting before the experiment 30 min. After exhaustion, skeletal muscle tissue samples were collected. Save immediately in liquid nitrogen.

1.2. RNA inhibition of interference in plateau pika Ldhc study on gene expression

Ldhc Gene ShRNA design of expression plasmid interference fragment with reference to [2]. According GenBank Reported plateau pikaLdhcGene'sCDNA Sequence (Hq704678), Select2.A target point (321~339 And 855~875), BlastSoftware for sequence homology analysis, period Intraperitoneal injection of adenovirus Pmul tirnai-ldhc Skeletal Muscle of plateau pika Ldhc Gene silencing effect. And using RNA structure 4.2 Prediction of its secondary structure. SiRNA Interference sequence is Ldhc 321, Ldh855.

1.2.1. Ldhc gene specificity ShRNA construction of expression vector and adenovirus packaging

With reference to [2], according to plateau pika and humans (Homo sapiens) LdhcCoding sequence of the gene, Design and Synthesis ShRNATarget sequence, using synthetic Single Chain ShRNA After annealing, the double chain product was recovered by agarose gel electrophoresis with low melting point. DNA Fragment, build Ldhc Gene specificity ShRNA Expression linearization plasmid carrier into competent cells selected positive cloning in medium in overnight culture of extraction plasmid sequencing identification insert sequence. According DNA Sequencing results will and the DesignShRNASequence100%Same of recombinant plasmid and empty plasmid respectively the adenovirus packaging. Sequence of synthesis and sequencing, plasmid of link and virus Packaging by Wuhan Jingsai biological limited the company complete.

1.3. Anti-fatigue experimental

Three group animal injection 7/d After Anti-fatigue experimental the swimming method different group plateau pika turn into round barrel in water temperature control in18℃ About swimming exhaustive time to animal and sank under the water shall prevail accurate record time.

1.4. Fluorescence PCR detection MRNA level of expression

Reference [2] Trizol reagent and (Invitrogen Life Technologies) Method extraction skeletal muscle group Texture total RNA Nucleic Acid Protein Content Detector Determination A260/A280Value (1.8 < A260/A280 < 2.0) And concentration (≥ 0.4 g/L),4 mμg Total RNA The Fast Quant RT Kit (Gdnase) (Tiangen) Kit preparation CDNA. Use has some plateau pika (*Ochotona curzoniae*) Ldha, Ldhb and Ldhc Gene Specific primers the fluorescence quantitative PCR. Ldha Gene of upstream primers sequenc Amplification sequence length220 BP. LdhbGene of 0.8 μL PCR forward primer, 0.8 μL PCR Reverse

primer,0.4 μl Rox reference DyeII, 2L cDNA, 6 μL R Nase-free DDH2.O. Reaction Conditions: 95C30 s;95C5 S,60C34 S,40A loop. Abi 7500Real-Time Fluorescence Quantitative PCRYi (USA) collect and analyze data.

1.5. Western blot detection of protein expression

With reference to [2], the total protein of skeletal muscle tissue was extracted with the total protein extraction kit. Piercetm BCA protein assay kit (Thermo Fisher Scientific, USA) Determination of total protein concentration. Take40 μgEgg WhiteSDS-After polyacrylamide gel electrophoresis, go0.22 PVDF Membrane. 5%Skim milk powder diluent closed at room temperature 2h, with the first Anti LDH-C, Abcam Rabbit monoclonal antibody company,1:10,000 Dilution; GAPDH, Abcam Monoclonal Antibodies, Inc, 1:4000 Dilution) 4. Incubate overnight; TBST Anti-rabbit with sheep after washing IgG (Goat anti-rabbit, Abcam Company, 1:2000 Dilution) Normal Temperature Incubation 2h and using TBST Wash. Adopted ECL Fluorescent kits (Thermo Fisher Scientific, USA) Exposure and gel imaging system (Bio-Rad, USA) Take pictures.

1.6. Lactate dehydrogenase (ldh) activity and lactic acid (ld) and atp determination of content

Make et al. [3] Methods, skeletal muscle tissue according to quality/Volume Ratio1:9. With 0.9%Saline ice bath homogenate, 4.C5000 r/min entrifugal 10 min Take supernatant. The colorimetric method in accordance with the kit (Nanjing built biological technology limited the company, China) (Operation detection organization lactate dehydrogenase (LDH) Activity and lactic acid (LD) Content. Reference make

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Catalina and [4]. The luciferase-Luciferase enzymatic in accordance ATP Kit (Jiangsu Pik Wan days biological technology limited the company, China) (Operation detection organization in ATP Content. Organization in accordance with Quality/Volume than 1:5 Proportion with kit in cracking liquid ice bath uniform Pulp, 4 ℃ 12,000 r/min Centrifugal 10 min Take supernatant. To ATP Concentration and cracking liquid protein concentration of ratio to said organization in ATP The relative content (Antibody mol/g).

1.7. Interference rate of calculation

Interference group interference Efficiency = (Blank control group average-interference group average)/Blank control group average shell group interference Efficiency = (Blank control group average-shell group average)/Blank control group average [5].

1.8. Data statistical analysis

"Excel 2007AndSPSSSoftware finishing data and analysis experimental data single factors variance analysis (One-way ANOVA) Data with average ± Standard Deviation said, $P < 0.05$For difference has statistical significance.

2. Results and analysis

2.1. Anti-fatigue experimental

Interference group, Shell Group and blank control group plateau pika (*Ochotona curzoniae*) exhaustive Swimming time in turn (749.00 ± 67.76) S, (843.90 ± 77.12) S And (855.47 ± 47.41) Shell Group and blank control group no significant difference ($P > 0.05$) Interference group swimming time was significantly lower than that of the Shell Group and blank control group ($P < 0.05$).

2.2. Plateau pika (ochotona curzoniae) skeletal muscle organization ldhcexpression Level

MRNALevel on plateau pika (Ochotona curzoniae) Skeletal Muscle organization in Ldha, Ldhb and Ldhc Gene were expression and blank control group compared interference group Ldha and Ldhb Gene of expression were no significant difference ($P > 0.05$) And LdhcGene expression very significantly reduce ($P < 0.01$) Adenovirus on Ldhc Gene of interference rate41.73%. In protein level plateau pika (*Ochotona curzoniae*) Skeletal Muscle organization in LDHA, LDHB And LDHC compared with the blank control group, the interference group Ldha And Ldhb with no significant difference in subunit expression ($P > 0.05$), And Ldhc subunit expression was significantly reduced (PLess than 0.01), Adenovirus pair LdhcGene interference rate is15.76%.

2.3. Lactate dehydrogenase in skeletal muscle (ldh) active and milk acid (ld) and tp content

Compared with the blank control group and the empty shell group, the lactate dehydrogenase (LDH) Activity and lactic acid (LD) And ATP Significantly reduced (PLess than 0.01), There was no significant difference between the empty shell group and the blank control group ($P > 0.05$), Adenovirus pair LDH, LD And ATP The interference rate is23.98%,51.08% And 19.29%.

3. Discussion

An [5] Direct Injection of adenovirus into skeletal muscle of plateau pika Pmultirnai-ldhc Compared with the blank control group MRNA and protein level LdhcGene expression was down 82.18% and 82.29% Lactate Dehydrogenase (LDH) Activity and lactic acid (LD), ATP The content also decreased 28.21%, 48.38% and 27.88% relatively. Sports Time has fallen 25.97%. An [5] in his experiments show that intraperitoneal injection0.65AdenovirusPmultirnai-ldhcCan fully enter the heart, liver and brain tissue, can significantly silence Ldhc Gene; in MRNA And protein level, in myocardial tissue Ldhc Gene expression was down 48.11% and 19.27%; Liver Tissue Ldhc Gene expression decreased 70.16% and 25.82%; Brain Tissue Ldhc Gene expression was down 49.08% and 25.36%. Lactate Dehydrogenase (LDH) Activity and lactic acid (LD) And ATP The content is also period intraperitoneal injection of adenovirus Pmultirnai-ldhc skeletal muscle of plateau Ldhc Gene of interference efficiency; F.Adenovirus Pmultirnai-ldhc LDHC Protein of interference efficiency [6]. In electrophoresis figure respectively representative Ldha, Ldhb,

LdhcGene and LDHC Protein of expression mode wich1–3 respectively representative interference group, Shell Group and blank control group. Organization. The Organization. $P < 0.01$. A. expression Level. Ldha mRNA; B. expression Level. Ldhb mRNA; C. expression Level. Ldhc mRNA; D. expression Level. LDHC; E. interference efficiency. pmultirnai-ldhc. Ldhc gene; F. interference efficiency. pmultirnai-ldhc. LDHC protein. figure a B C, D, electrophoresis: represent. Expression Pattern. Ldha Ldhb Ldhc MRNA, LDHC protein. 1, 2, 3 represent RNAi-Ldhc RNAi-HK, control. Organization [7,8]. The Organization. $P < 0.01$.

Adenovirus Pmultirnai-ldhc The plateau pika (Ochotona curzoniae) Skeletal Muscle organization inLDHActivity of influence; B.Adenovirus Pmultirnai-ldhc The plateau pika (*Ochotona curzoniae*) Skeletal Muscle organization in LDH live of the interference efficiency; C. Adenovirus Pmultirnai-ldhc The plateau pika (*Ochotona curzoniae*) Skeletal Muscle organization in LDOf influence; D. Adenovirus Pmultirnai-ldhc. The plateau pika (*Ochotona curzoniae*) Skeletal Muscle organization in LD. The interference efficiency; E. Adenovirus Pmultirnai-ldhcThe plateau pika (*Ochotona curzoniae*) Skeletal Muscle organization in ATP Of influence; F. Adenovirus Pmultirnai-ldhc the plateau pika (*Ochotona curzoniae*) Skeletal Muscle Organization ATP The interference efficiency. The Organization. $P < 0.01$. effect of pmultirnai-ldhc on the activity of LDH in plateau pika skeletal muscle; B. conference level on the LDH in plateau pika skeletal muscle; C. effect of pmultirnai-ldhc on the activity of LD in plateau pika skeletal muscle; D. conference level on the LD in plateau pika skeletal muscle; E. effect of pmultirnai-ldhc on the activity of ATP in platform pika skeletal muscle; F. conference level on the ATP in plateau pika skeleton muscle [9.10]. Takayasu $P < 0.01$.

3. Period Intraperitoneal injection of adenovirus Pmultirnai-ldhc Skeletal Muscle of plateau pika LdhcGene silencing effect 445 Significant decrease, in which the myocardial tissue decreased 25.58%,41.94%,21.23%, Respectively, in liver tissue 28.16%, 15.90%, 24.66%. In the brain tissue16.65%,12.78%,18.50%. In this study, intraperitoneal injection of adenovirus into plateau pika Pmultirnai-ldhc 7d After, skeletal muscle tissue Ldhc Gene in MRNA. And protein table levels were significantly decreased, respectively decreased41.73%And15.76%Lactate Dehydrogenase (LDH) activity and lactic acid (LD) ATP The content 23.98%, 51.08% and 19.29% relatively. Sports Time has fallen 12.45% [11,12]. And Wang (2017) showed the results of intraperitoneal injection can significantly silence myocardial, liver, brain and bone muscle tissue Ldhc Gene expression, indicating that intraperitoneal injection SiRNA. It can significantly silence gene expression in tissue cells, indicating that intraperitoneal injection can achieve the effect of silencing.

Conflict of interest: The author declares no conflict of interest.

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