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ORIGINAL ARTICLE

Intraperitoneal Injection Adenovirus Pmultirnai-ldhc Okay Plateau Pika (Ochotona curzoniae) Skeletal Muscle Ldhc Gene of Silence Effect

Yangzhi Deng

Xi'an Jiaotong University, Shijiazhuang 050100

Abstract:Intraperitoneal injection is a kind of simple and convenient to drug of style in order to verify intraperitoneal injection adenovirusPmultirnai-ldhcThe plateau pika (Ochotona curzoniae (Ochotona curzoniac) Skeletal MuscleLdhcGene Silence of feasibility will27Only 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 mLAdenovirusPmultirnai-ldhcAnd adenovirusPmultirnai-NSBlank control group injected with the same amount of physiological saline injection after7/dDetection Skeletal Muscle inLdhcGeneMRNAAnd 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 inMRNAAND PROTEIN LEVEL,LdhcGene expression respectively reduce41.73%And15.76%; Lactate dehydrogenase (LDH) Activity, lactic acid (LD) AndATPContent respectively reduce23.98%,51.08%And19.29%. Results (intraperitoneal injection adenovirusPmultirnai-ldhcCan effective silence Skeletal Muscle inLdhcGene 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.2012); Duan Yinan (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 (Guo Hua, *et al*.2008), Which also causes drug depletion and reduces the amount of drugs entering the blood. Macromolecular adenovirusPmultirnai-ldhcAfter intraperitoneal injection, the liver can effectively reach the skeletal muscle and play a role.7 dEffects of Adenovirus on Skeletal Muscle of plateau pikaLdhcGene 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 size27Only, weight150 ~ 200g, Randomly divided

3. Groups, per group9.Only. No1.Group as interference group (RNAi-ldhc), Intraperitoneal injec-

tion4x10^{9}·PFU(The number of virus particles)0.65AdenovirusPmultirnai-ldhc2.2.Groups are empty groups (RNAi-HK), Injecting the same dose of adenovirusPmultirnai-NS2.3.Control group (Control), Injecting the same dose of saline. Plateau pika kept after injection7 d, Resting before the experiment30 minAfter exhaustion, skeletal muscle tissue samples were collected.

Save immediately in liquid nitrogen.

1.2 RNAInhibition of interference in plateau pikaLdhcStudy on Gene Expression

1.2.1 LdhcGeneShRNADesign of expression plasmid interference FragmentWith reference to Wang Zhijie (2017), AccordingGenBankReported plateau pikaLdhcGene'sCDNASequence (Hq704678), Select2.A target point (321 ~ 339And855 ~ 875),BlastSoftware for sequence homology analysis,

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And usingRNA structure 4.2Prediction of its secondary structure.SiRNAInterference sequence isLdhc 321(5′-ttt gtt agt act tca aag att act TCA aga cgg taa tct ttg aag tac taa tt ttt ′)

Ldh855(5′-cct cgc tat tgg act gtc gat TCA aga CCTA aga cag tcc aat agc ttt ′).

1.2.2 LdhcGene specificityShRNAConstruction of Expression Vector and adenovirus Packaging

With reference to Wang Zhijie (2017), According to plateau pika and humans (Homo sapiens)LdhcCoding sequence of the gene, Design and SynthesisShRNATarget sequence, using synthetic Single ChainShRNAAfter annealing, the double chain product was recovered by agarose gel electrophoresis with low melting point. DNAFragment, buildLdhcGene specificityShRNA

Expression linearization plasmid carrier into competent cells selected positive cloning in medium in overnight culture of extraction plasmid sequencing identification insert sequence. AccordingDNASequencing 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 jing sai biological limited the company complete.

1.3 Anti-fatigue experimental

Three group animal Injection7/dAfter 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 FluorescencePCRDetectionMRNALevel of expression

Reference Wang Zhi clean and (2017)Trizol reagent and(Invitrogen Life Technologies) Method extraction skeletal muscle groupTexture totalRNANucleic Acid Protein Content detector DeterminationA_{260}/A_{280}Value ($1.8 < A_{260}/A_{280} < 2.0$) And concentration (≥0.4g/L),4 mu gTotalRNATheFast Quant RT Kit(. Gdnase)(Tiangen) Kit preparationCDNA. Use has some plateau pika (Ochotona curzoniae)Ldha,LdhbAndLdhcGene Specific primers the fluorescence quantitativePCR.LdhaGene of upstream primers sequence5 '-TTg gtc cag cgg aat GTA-3 ′Downstream primers sequence5 '-ggt gaa ctc cca gcc TTT-3 ′Amplification sequence length220 BP.LdhbGene of upstream primers sequence5 '-TGT TGG ACA

Agt cgg aat G-3 ′Downstream primers sequence5 '-ctg aag aaa cag gct CCC-3 ′; Length139 BP.LdhcGene of upstream primers sequence5 '-CAGGag gga gaa GGT-3 ′Downstream primers sequence5 '-atg aca cga gag gca ggt AA-3 ′; Length 179 bp. Referenceβ-actinGene of upstream primers sequence 5 '-ctc ttc cag ccc tcc ttc TT-3 ′Downstream primers sequence5 '-agg tcc tta cgg atc tcc ′; Length is98 bp. FollowSYBR®Premier exTaq™II (TLI rnaseh plus)(Takara) Kit description preparation reaction system:10 l sybr @ premix ex?TaqII,0.8 μL PCR forward primer,0.8 μL PCR Reverse primer,0.4 μl Rox reference DyeII,

2 L cDNA?,6 μl RNase-free DDH_2O. Reaction Conditions:95C30 s;95C5 S,60C34 S,40A loop.Abi 7500Real-Time Fluorescence QuantitativePCRYi (USA)

Collect and analyze data.

1.5 Western blotDetection of Protein Expression

With reference to Wang Zhijie (2017), 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 PVDFMembrane.

5%Skim milk powder diluent closed at room temperature2 h, With the first Anti ()

LDH-C,AbcamRabbit monoclonal antibody company,1.：10 000

Dilution;GAPDH,AbcamMonoclonal Antibodies, Inc,1.：4 000Dilution)4.Incubate overnight;TBSTAnti-rabbit with sheep after washingIgG(Goat anti-rabbit,AbcamCompany,1.：2 000Dilution) Normal Temperature Incubation2 hAnd usingTBSTWash. AdoptedECL

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) AndATPDetermination of Content

Reference shulina *et al* (2015) Methods, skeletal muscle tissue according to quality/Volume Ratio1.：9.With0.9%Saline ice bath homogenate,4.C5 000 r/minCentrifugal10 minTake 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 (2015) The luciferase-Luciferase enzymatic in accordanceATPKit (Jiangsu Pik Wan days biological technology limited the company, China) (Operation detection organization inATPContent. Organization in accordance with Quality/Volume than1：5Proportion with kit in cracking liquid ice bath uniform Pulp,4 ℃ 12 000 r/minCentrifugal10 minTake supernatant. ToATPConcentration and cracking liquid protein concentration of ratio to said organization inATPThe 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 (Wang Zhi clean and2017).

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)SAnd (855.47 ± 47.41)SShell 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

($P<0.05$Figure1).

2.2 Plateau pika (Ochotona curzoniae) Skeletal Muscle OrganizationLdhcExpression Level

MRNALevel on plateau pika (Ochotona curzoniae) Skeletal Muscle organization inLdha,LdhbAndLdhcGene were expression and blank control group compared interference groupLdhaAndLdhbGene of expression were no significant difference (P> 0.05) AndLdhcGene expression very significantly reduce (P<0.01) Adenovirus onLdhcGene of interference rate41.73%. In protein level plateau pika (Ochotona curzoniae) Skeletal Muscle organization inLDHA,LDHBAndLDHCCompared with the blank control group, the interference groupLdhaAndLdhbNo significant difference in subunit expression (P> 0.05), AndLdhcSubunit expression was significantly reduced (PLess than 0.01), Adenovirus pairLdhcGene interference rate is15.76%(Figure2.).

2.3Lactate Dehydrogenase in skeletal muscle (LDH) Active and milk

Acid (LD) AndTPContent

Compared with the blank control group and the empty shell group, the lactate dehydrogenase (LDH) Activity and lactic acid (LD) AndATPSignificantly 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,LDAndATPThe interference rate is23.98%,51.08%And19.29%(Figure3.).

3. Discussion

An(2017) Direct Injection of adenovirus into skeletal muscle of plateau pikaPmultirnai-ldhcCompared with the blank control groupMRNAAND PROTEIN LEVELLdhcGene expression was down82.18%And82.29%Lactate Dehydrogenase (LDH) Activity and lactic acid (LD),ATPThe content also decreased.28.21%,48.38%And27.88%Sports Time has fallen25.97%. Wang Zhijie (2017) Experiments show that intraperitoneal injection0.65AdenovirusPmultirnai-ldhcCan fully enter the heart, liver and brain tissue, can significantly silenceLdhcGene; inMRNAAnd protein level, in myocardial tissueLdhcGene expression was down48.11%And19.27%; Liver TissueLdhcGene expression decreased

70.16%And25.82%; Brain TissueLdhcGene expression was down49.08%And25.36%Lactate Dehydrogenase (LDH) Activity and lactic acid (LD) AndATPThe content is also

3.Period Intraperitoneal injection of adenovirusPmultirnai-ldhcSkeletal Muscle of plateau

LdhcGene of interference efficiency;F.AdenovirusPmultirnai-ldhcOkayLDHCProtein of interference efficiency. FigureA,B,CAndDIn electrophoresis figure respectively representativeLdha,Ldhb,LdhcGene andLDHCProtein of expression mode which1,2And3Respectively 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.LdhaLdhbLdhcMRNA, LDHC protein.. 1 2, 3 represent RNAi-ldhc RNAi-HK, control.Organization. The Organization.P<0.01. In ·444In · Zoology magazineChinese Journal. Zoology 53Volume

a. AdenovirusPmultirnai-ldhcThe plateau pika (Ochotona curzoniae) Skeletal Muscle organization inLDHActivity of influence;B.AdenovirusPmultirnai-ldhcThe plateau pika (Ochotona curzoniae) Skeletal Muscle organization inLDHLive

Of the interference efficiency;C.AdenovirusPmultirnai-ldhcThe plateau pika (Ochotona curzoniae) Skeletal Muscle organization inLDOf influence;D.AdenovirusPmultirnai-ldhcThe plateau pika (Ochotona curzoniae) Skeletal Muscle organization in

LDThe interference efficiency;E.AdenovirusPmultirnai-ldhcThe plateau pika (Ochotona curzoniae) Skeletal Muscle organization inATPOf influence;F.AdenovirusPmultirnai-ldhcThe plateau pika (Ochotona curzoniae) Skeletal Muscle Organization ATPThe interference efficiency.Organization. The Organization.P<0.01.

A. 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.TakayasuP<0.01.

3.Period Intraperitoneal injection of adenovirusPmultirnai-ldhcSkeletal Muscle of plateau pikaLdhcGene 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 pikaPmultirnai-ldhc 7 dAfter, skeletal muscle tissueLdhcGene inMRNAAnd protein table

Levels were significantly decreased, respectively decreased41.73%And15.76%Lactate Dehydrogenase (LDH) Activity and lactic acid (LD) ATPThe content 23.98%,51.08%And19.29%Sports Time has fallen 12.45%. And Wang Zhijie (2017) The results of intraperitoneal injection can significantly silence myocardial, liver, brain and bone

Muscle tissueLdhcGene expression, indicating that intraperitoneal injectionSiRNAIt can significantly silence gene expression in tissue cells, indicating that intraperitoneal injection can achieve the effect of silencing.

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