2007 广东省体育科学研究论文选

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杨迺军 许宗祥 主编

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北京体育大学出版社

责任编辑 木 凡 审稿编辑 鲁 牧 责任校对 冬 梅 责任印制 陈 莎

图书在版编目(CIP)数据

2007 广东省体育科学研究论文选/杨迺军,许宗祥主编 . - 北京:北京体育大学出版社,2007.11 ISBN 978 -7 -81100 -876 -0

Ⅰ.2… Ⅱ.①杨…②许… Ⅲ. 体育 - 科学研究 - 文 集 IV. G80-53

中国版本图书馆 CIP 数据核字(2007)第 174801 号

2007 广东省体育科学研究论文选

杨迺军 许宗祥 主编

出 版 北京体育大学出版社

地 址 北京海淀区中关村北大街

XX tit www. bsup, en

фß 编 100084

发 行 新华书店总店北京发行所经销

ED 刷 北京市昌平阳坊精工印刷厂

开 本 787×960 毫米 1/16

ED 张 14.5

> 2007年11月第1版第1次印刷 定 价 29.00元

(本书因装订质量不合格本社发行部负责调换)

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贺 词

各位代表、同志们:

正当全省人民深入贯彻落实党的十七大会议精神之际, 我们迎来了广东省体育科学学会第二届体育科学大会的召 开。这是我省体育界的一次盛会,也是全省人民的一件大 事。首先,我代表省体育局、省体育科学学会,向大会的召



开表示热烈的祝贺! 向全体代表和全省体育科技工作者, 致以亲切的问候!

学会是党领导下的人民团体,是科技工作者的群众组织。改革开放以来特别是党的十六大以来,广东省体育科学学会坚持围绕"奥运争光、竞技体育、全民健身"的中心服务大局,团结和组织广大体育科技工作者,积极主动地面向群众体育、竞技体育和体育产业主战场,在促进科技与体育的结合、促进科学技术的普及和推广、促进科技人才的成长和提高等方面发挥了重要作用,为建设体育强省、争当全国体育排头兵作出了积极贡献。

广东今后的体育发展关键在科技,根本在人才。我们必须高度重视科技,高度 重视人才,高度重视提高全省人民的体育科技文化水平。充分发挥科技人才的积极 性、主动性、创造性,是建设创新型广东体育的战略举措。希望我省体育科学学会在各级党委、政府的领导下,认真履行职责,继续做好学会工作,进一步发挥学会在团结科技人才、为"奥运争光、竞技体育、全民健身"提供科技服务和促进全民科学素质提高等方面的作用,为推进我省科技创新、加快我省体育的建设作出新的更大的贡献。

预祝大会圆满成功!

广东省体育局局长

广东省体育科学学会名誉理事长

2007年11月23日

前 言

为了深入贯彻党在十七大提出的"科学发展观"精神,全面展示广东省体育科学研究的最新成果,发挥体育科学的理论指导作用,加强广东省竞技体育与群众体育科技工作者的交流,进一步推动广东省科技兴体的科学化进程,同时为2008年奥运会提供科学支持,广东省体育科学学会于2007年11月23日至25日在广州体育学院举行了第二届体育科学大会论文报告会。

本届科学大会以"21世纪的体育科学与和谐社会"为主题,以反映广东省体育科学发展对社会和谐发展的作用。大会征集了来自广东省各院校及研究机构的论文近百篇,论文水平较往年明显提高,其内容既有理论探索,又有实践总结。投稿作者年轻化,其中部分是优秀的在读的硕士生和博士生,其论文质量较高。经过各领域的专家严格、公平地评审,本次大会共录用论文79篇,各学科录取论文数分别为:人文社科类25篇,自然科学类54篇;并评出一等奖1篇,二等奖7篇,三等奖13篇,青年奖2篇。

本论文集所收录的论文把握了国内外体育科研的最新动态,涉及运动训练学、运动医学、运动生理学、运动生物化学、运动营养学、运动生物力学、运动心理学、体育社会学、体育经济学、体育管理学、体育信息学等体育科学研究的各个领域,体现了当前体育学科与其他学科交叉、融合的发展趋势。这些论文是近年来广东省体育科研成果的优秀代表,我们结集出版这些论文,希望能加强体育科技工作者之间的交流,促进广东体育科学研究的发展。

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包燃科学类

1

Low Intensity Laser Irradiation Improve the Mitochondrial Dysfunction of C2C12 Induced by Electrical Stimulation

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ABSTRACT

We investigated the effects of ES and LIL on the mitochondrial function of cultured C2C12 myotubes in order to find the dosage which can be used to improve the function of mitochondria and then rehabilitate exercise-induced fatigue or damage. Cultured C2C12 myotubes were subjected to ES or/and LIL irradiation at various intensity. ROS was detected with DCFH fluorescent probe and mitochondrial function was assessed with MTT. The results showed that ES at 20ms \$\, 5Hz\$ and 45V intensity for 75 min can induce mitochondrial dysfunction of cultured C2C12 myotubes. ES-induced mitochondrial dysfunction were improved and degenerated by LIL at 0.33 \$\pi 8.22 \text{J/cm}^2\$ and 11.22 \$\pi 14.16 \text{J/cm}^2\$, respectively. The improvement was significantly higher with LIL at 0.33 and 1.34 \text{J/cm}^2\$, respectively. We conclude that treatment of the cells with proper dosage of LIL irradiation significantly diminished ROS production and rehabilitate mitochondrial function which may provides a foundation for PBM on exercise induced mitochondrial dysfunction or skeletal muscular fatigue.

KEYWORDS: C2C12 myotubes; electrical stimulation; low intensity laser; reactive oxygen species; mitochondrial function

INTRODUCTION

Many research had shown that exercise stress can induce rapid increase of reactive

oxygen species (ROS), and then the dysfunction of the cells and tissue, especially the dysfunction of mitochondria. Fatigue are related with changes in muscle function and local biochemical changes, which occur during high-intensity exercise or titanic stimulation.

Electrical stimulation (ES) can simulate the contraction of skeletal muscle. Much work had been done in the field of the research on the function of C2C12 myotubes by means of ES and ROS increasement and mitochondrial dysfunction, the resultant of ES, can simulate muscular fatigue. In this trial, we used the cell model as usually used in the world to deep study the mechanism of PBM.

Low intensity laser (LIL) as a novel therapy form has been widely used in clinical trials in recent years, though its biological mechanisms remain unclear. Mitochondria is very sensitive to monochromatic light or LIL, LIL has been found to modulate various biological processes, such as increasing mitochondrial respiration and ATP synthesis, modulating the function of cell membrane, inducing various changes of mitochondrial enzyme, eliminating O2-. and enhancing the activity of SOD.

Photobiomodulation (PBM) is the hot topic in the field of laser medicine. Reseach had shown that PBM can modulate many kind of tissues and produce different cellular or subcellular effects, including the healing effects of the decreased cell activity, so it can be called a green sports medicine method. Recent study had found LIL could reduce muscular fatigue during titanic contractions in rats and LIL had no effect on the recovery of damage though it could alleviate pain induced by DOMS.

There are two key criteria to successfully invoking PBM effect: one is the appropriate application of energy of which if excessive amounts are applied, tissue destruction occurs; another is the wavelength of the laser and it is critical for the two primary reasons: It determines determines the depth of photon penetration. Whereas muscle exist underneath the skin, LIL should have a certain depth of penetration. 810nm laser had powerful depth of penetration, so we anticipate it be an important therapy in vitro for exercise induced muscular damage and fatigue. However, PBM of low level near infrared on myotubes and their mitochondrial dysfunction was little studied.

In this paper, the effects of low intensity 810nm Ga-Al-As diode laser irradiation (LIL) on ES-induced mitochondrial dysfunction of C2C12 myotubes will be studied, and aims to investigate the effect of laser radiation (810 nm, radiant exposure at 0.33 \sim 14.16 J/cm²) on the mitochondrial function and metabolism of free radical so as to offer

some theoretical basis of PBM on clinical application.

EXPERIMENTAL MATERIALS AND METHODS

Cell culture

C2C12 cells were bought from Institute of Basic Medical Sciences of China. Cells were cultured in growth medium (GM; Dulbecco's modified Eagle's medium [DMEM] containing 10% fetal bovine serum (FBS), 100 IU/ml penicillin and 100 µg/ml streptomycin) in a humidified incubator at 37°C with 5% CO₂. To induce differentiation, nearly confluent C2C12 cells were incubated in DMEM containing 2% heat-inactivated horse serum (differentiation medium; DM) for six days.

Electrical stimulation protocol

The design of the electrical stimulation apparatus was derived by modifying existing methods. Myotubes after differentiated for six days were stimulated at a frequency of 5 Hz and an intensity of 45 V using electrical stimulator (TY-C type). Stimulation were performed acutely for 15, 30, 45, 60, 75, 90, 105, 120, 150 or 180 min separately in experimental group. Cell extracts obtained from each dishes were prepared immediately after stimulation.

LED Irradiation

Cultured myotubes are irradiated continuously by a low intensity 810nm Ga-Al-As diode laser (LIL). The doses varied from (0. 33 \sim 14. 16) J/cm² over a period of 600s. Irradiation was performed through a fiber so that the homogeneous light spot totally covered the bottom of the dishes (9. $6 \, \text{cm}^2$). Intensity measurements were measured by an optical power meter made in Advantest Company, model TQ8210. Irradiation was performed at room temperature in the dark.

ROS Measurements

A fluorescent probe, 2', 7'-dichlorofluorescin diacetate (DCFH-DA), was used for the assessment of intracellular ROS formation in cultured C2C12 myotubes. This assay is a reliable method for the measurement of intracellular ROS such as hydrogen peroxide ($\rm H_2O_2$), hydroxyl radical (OH \cdot), and hydroperoxides (ROOH). Myotubes were loaded with the probe and incubated for 20min. After loading, the cells were washed for electrically stimulation or/and LIL irradiation, and then the samples were collected immediately and placed in the spectrofluorimeter to determine the ROS production at the excitation and emission wavelength of 522nm and 488nm?. The peak value of the luminescence

as a function of time was taken after subtracting the value of the blank signal (luminescence of the illuminated medium).

Total mitochondrial function assays (MTT)

The total mitochondrial function of myotubes was assessed by the MTT (3- (4, 5-dimethylthiazol-2-yl) -2, 5, -diphenyltetrazolium bromide; Sigma-Aldrich) assay. At the indicated times, MTT was added to the cells at a final concentration of 0. 5 mg/ml for 4 hours of incubation at 37°C , 5% CO₂ incubator. Absorbance values were determined with enzyme micro-plate reader (EL311S, Bio-Tek Instruments) with detection is fixed at $570 \, \text{nm}$.

Statistical Analysis

All data are shown as mean \pm SD. Significance was determined with One-way ANO-VA. A value of P < 0.05 was considered to be significant.

RESULTS

Experiments on C2C12 Myotubes Exposed to Electrical Stimulation

There was a "double-apex curve" peaking at 75min and 120min after six days stimulation for the curve of ES-induced ROS in C2C12 myotubes vs stimulation periods, which indicated that ES could induce a rapid increase of ROS, and its production varied with stimulation periods. The curve of OD values of MTT vs stimulation periods showed that mitochondrial function declined and then improved after being stimulated for 75min and 90 min, respectively. The results as shown in Table. 1 and 2 indicated that ES75min at 45V, 20ms and 5Hz intensity can induce mitochondrial dysfunction of cultured C2C12 myotubes.

Effects of LIL on the ROS production of C2C12 myotubes

Different dosage of LIL has different effects on ROS production of the differentiated C2C12 myotubes. ROS production increased when irradiated at the dosage of (5. 358 \sim 14. 16) J/cm² in pure LIL Group, while there were no difference at the dosage below 5. 358J/cm² compared with the control Group (as shown in Fig1. A). In ES + LIL Group (Fig1. B), ROS production was significantly inhibited and promoted by LIL after being irradiated at 0.33J/cm² and 14.16 J/cm², respectively (P < 0.01) and had the same tendence as shown in Fig1. A.

Effects of LIL on the mitochondrial dysfunction of C2C12 myotubes

There were no changes of mitochondrial function when normal cells were irradiated at