計畫名稱：肌肉缺血再灌流損傷分子機轉

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一、中文摘要

肌肉缺血再灌流傷害會造成肌肉移植後肌肉功能不全或因血管阻塞後再灌流造成 compartment syndrome。我們在大鼠大腿處以 tourniquet 造成 4 小時缺血，再以 in vivo MCLA 冷光測定儀測定不同時間再灌流所造成 oxygen free radicals (ORF) 量，發現所測得最高量在第二天，此時以抗中性球抗體(MCA967)染出最高量的中性球數，以及以 TUNEL 染出最多的肌肉細胞凋亡。故我們應尋求較長期(>2d)有效的 ORF 清除劑，尤其是中性球的聚集因子(chemokine)的去除，才可有效的避免肌肉缺血再灌流傷害。

關鍵詞：muscle; oxygen free radicals; ischemia reperfusion injury

Abstract

We studied the ischemia reperfusion injury of skeletal muscle including oxygen free radicals (OFRs) detection with MCLA chemiluminescence, neutrophil staining and TUNEL. The OFRs as well as neutrophils and muscle cell apoptosis peaked by day 2 after release of tourniquet. These findings are interesting because the neutrophil number and the muscle cells showing apoptosis are also peaking on day 2. Neutrophils are likely the major source of free radicals. This piece of information and this animal model are helpful for designing and evaluating anti-oxidant regimens for decreasing ischemia reperfusion injury of skeletal muscles.

Keywords: muscle; oxygen free radicals; ischemia reperfusion injury

二、Introduction

Ischemia-reperfusion injury (IRI) can be a significant factor affecting the outcome of microvascular free muscle transfer or in the compartment syndrome of extremities. Reperfusion after a transient ischemia often causes greater injury to the affected organs than ischemia itself. Upon reperfusion, increased amount of xanthine oxidase metabolize xanthine and hypoxanthine, which are metabolites of ATP during ischemia, to produce oxygen free radicals (OFRs). Although superoxide dismutase (free radical scanvenger) or alloputinol (xanthine oxidase inhibitor) are partially effective in reducing injury at this early stage, it is clear that neutrophils adhered to the postcapillary venules release even more OFRs and destructive proteases.

The microvascular endothelium plays an important role in initiating the events associated with IRI by releasing chemotactic factors. Many cells are known to produce chemokines after being stimulated with pro-inflammatory stimuli. These chemikines could recruited differenet subsets of leukocytes as needed. However, in IRI , too many activated neutrophils accumulated into ischemic tissue might turn out to be harmful. There were reports about the expression of cytokine-induced neutrophil chemoattractant (CINC), which attract neutrophils in rat brain IRI. The chemokines in skeletal muscle IRI are still unknown. We had experience dissecting the chemokine profile (Two C-X-C chemokines: KC and IP-10, three C-C chemokines: MCP-1, MIP-1 alpha, and RANTES) in degenerating peripheral nerves. Hypothesizing that CINC mRNA might increase early in the course of IRI of rat skeletal muscle, we tested for the expression of CINC mRNA by quantitative reverse transcription-coupled PCR (RT-PCR), *in situ* hybridization, and for the presence of the CINC protein and neutrophils by immunocytochemistry.

There are many ways of measuring OFRs, but all have to deal with their short half-lives. The most common form of measurement are those of the products of OFRs attack on lipid, such as malondialdehyde (MDA). The thiobarbituric acid (TBA) test for MDA is non-specific. Many data of this method are difficult to interpret and compare. With the advent of very sensitive photon counting technique, ultraweak chemiluminescence (CL) from OFRs and enhanced with luminol, leucigenin or MCLA could be easily detected. A sensitive both *in vitro* and *in vivo* CL detecting system by Tohoku Co. Japan were recently available at our common lab. We would use a rat hind limb ischemia model by tourniquet to create 4 h ischemia, followed by various time of reperfusion.

The CL readings from exposed medial gastrocnemus muscle *in vivo* will be correlated with the following parameters to test the hypotheses: 1) OFRs are the main source of damage, and 2) the main source of OFRs is the neutrophils. The other three parameters are 1) tissue viability as stained with nitroblue tetrazolium, 2) neutrophils density within the venules or outside the venules as stained with anti-neutrophil antibody (MCA 967, Serotec, UK), 3) apoptosis as studied with DNA ladder on gel electrophoresis and TUNEL reaction.

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四、Results

1. Animal model

We created this model by cathetering the iliac arteries via either contralateral femoral artery or ipsilateral jugular artery till the bifurcation of iliac arteries, where the enhancer MCLA was injected totally into the ischemic side. The constant readings were got due to this method as well as accurate dose of MCLA.

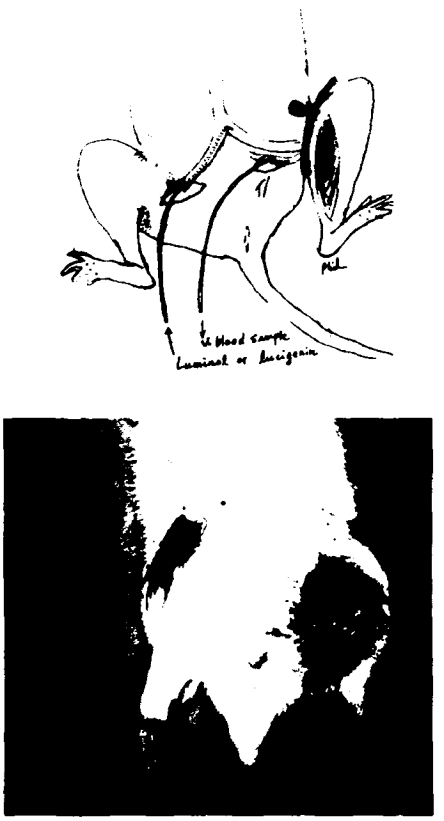

Fig. 1 An animal model of ischemia reperfusion injury of gastrocnemus muscle. The MCLA was infused through a catheter in the iliac artery near the bifurcation.

2. MCLA Chemiluminescence detection

By applying in vivo continuous measuring of MCLA chemiluminescence, we accidentally found the peak was not in the early phasse of reperfusion, but rather at 2 day. This is why most of the OFR scavengers work not so good, since most of the drug were used in the early phase.

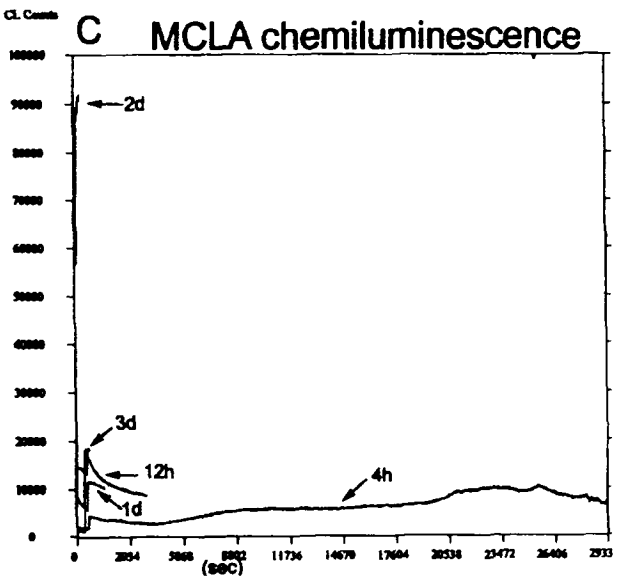

Fig.2 Intermittent CL measurement of gastrocnemus muscle in a representative animal after ischemia for 4 h and reperfused for various time periods. MCLA was infused at a speed of 0.125 mg/hr. The peaked measurement was on day 2.

3. Neutrophil and TUNEL staining

With fresh tissue on cryostat section, we developed neutrophil staining using Serotec's MCA967 antibodies. With paraformaldehyde fixed tissue, TUNEL was done, constantly showing apoptotic muscle cells, especially at 2 day.

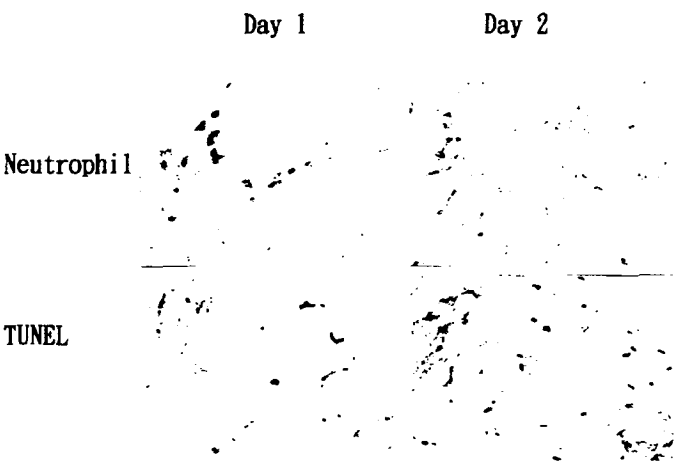

Fig.3 Upper panel showed the neutrophil- antibody immunoreactive cells on day 1 and day 2 . Neutrphils were less on day 1, day 3 or day 4 (not shown) when compared with that of day 2.Lower panel showed TUNEL positive nuclei of muscle cells on day 1 and day 2. There were more neutrophil infiltration and apoptotic muscle cells on day 2.

四. 討論及自我評估

The real-time oxygen free radical monitoring in reperfused skeletal muscle was done here, which to our surprise, the OFR peaked on the 2nd day. This piece of information is important since the regimen decreasing the OFRs must be prolonged after 2nd day. Otherwise the clinical result must be poor. The source of this OFR was under investigated by neutrophil staining and real-time PCR of CINC, which was preliminarily shown to be correlated. Although promising, the final result will be reached in recent months. Statistical evaluation was under way.

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