Eur J Vasc Endovasc Surg (2015) 49, 306–313

Protective Effect of Focal Adhesion Kinase against Skeletal Muscle Reperfusion Injury after Acute Limb Ischemia

M. Flück [a,b,*,c], **R.S. von Allmen** [b,c], **C. Ferrié** [a,b], **H. Tevaearai** [b], **F. Dick** [b]

[a] Balgrist University Hospital, University of Zurich, Switzerland
[b] Department of Cardiovascular Surgery, Inselspital, Bern University Hospital and University of Bern, Bern, Switzerland

WHAT THIS PAPER ADDS

Activation of focal adhesion kinase (FAK) is known to protect heart muscle against ischemia reperfusion (IR) injury. It is shown that IR-induced rhabdomyolysis, macrophage infiltration, and apoptosis of skeletal muscle are fiber type-specific phenomena graded by mitochondria reserves. Moreover, over expression of FAK protected fast skeletal muscle against IR injury by exploiting these reserves, possibly resulting in reduced tissue loss during IR. These findings may lead to novel therapeutic approaches to acute limb ischemia.

Objectives: In cardiac muscle, ischemia reperfusion (IR) injury is attenuated by mitochondrial function, which may be upregulated by focal adhesion kinase (FAK). The aim of this study was to determine whether increased FAK levels reduced rhabdomyolysis in skeletal muscle too.

Material and methods: In a translational *in vivo* experiment, rat lower limbs were subjected to 4 hours of ischemia followed by 24 or 72 hours of reperfusion. FAK expression was stimulated 7 days before (via somatic transfection with pCMV-driven FAK expression plasmid) and outcomes were measured against non-transfected and empty transfected controls. Slow oxidative (i.e., mitochondria-rich) and fast glycolytic (i.e., mitochondria-poor) type muscles were analyzed separately regarding rhabdomyolysis, apoptosis, and inflammation. Severity of IR injury was assessed using paired non-ischemic controls.

Results: After 24 hours of reperfusion, marked rhabdomyolysis was found in non-transfected and empty plasmid-transfected fast-type glycolytic muscle, tibialis anterior. Prior transfection enhanced FAK concentration significantly ($p = 0.01$). Concomitantly, levels of BAX, promoting mitochondrial transition pores, were reduced sixfold ($p = 0.02$) together with a blunted inflammation ($p = 0.01$) and reduced rhabdomyolysis ($p = 0.003$). Slow oxidative muscle, m. soleus, reacted differently: although apoptosis was detectable after IR, rhabdomyolysis did not appear before 72 hours of reperfusion; and FAK levels were not enhanced in ischemic muscle despite transfection ($p = 0.66$).

Conclusions: IR-induced skeletal muscle rhabdomyolysis is a fiber type-specific phenomenon that appears to be modulated by mitochondria reserves. Stimulation of FAK may exploit these reserves constituting a potential therapeutic approach to reduce tissue loss following acute limb IR in fast-type muscle.

Article history: Received 20 April 2014, Accepted 15 November 2014, Available online 30 December 2014

Keywords: Reperfusion injury, Ischemia, Focal adhesion kinase, Electroporation, Gene transfer

INTRODUCTION

Acute limb ischemia is among the most common peripheral vascular emergencies.[1,2] It threatens tissue viability even after a relatively short insult by ischemia reperfusion (IR) associated inflammation and rhabdomyolysis.[3] Apoptotic cell death is a programmed, energy dependent process, which is believed to play a pivotal role in the tissue response to IR. A variety of death signals including calcium overload[4] may trigger apoptosis or inflammation via transition pore-mediated destruction of mitochondria (Fig. 1A). Bcl-2 family molecules, such as BAX and Blc-2, which either promote or inhibit transition pore opening, exert a critical role on whether a cell will live or die.[5] The involvement of

DOI of original article: http://dx.doi.org/10.1016/j.ejvs.2014.12.020
[c] These authors contributed equally to this work.
* Corresponding author. M. Flück, Laboratory for Muscle Plasticity, Balgrist University Hospital, Department of Orthopedics, University of Zurich, Forchstrasse 340, 8008 Zurich, Switzerland.
E-mail address: mflueck@research.balgrist.ch (M. Flück).

http://dx.doi.org/10.1016/j.ejvs.2014.11.011

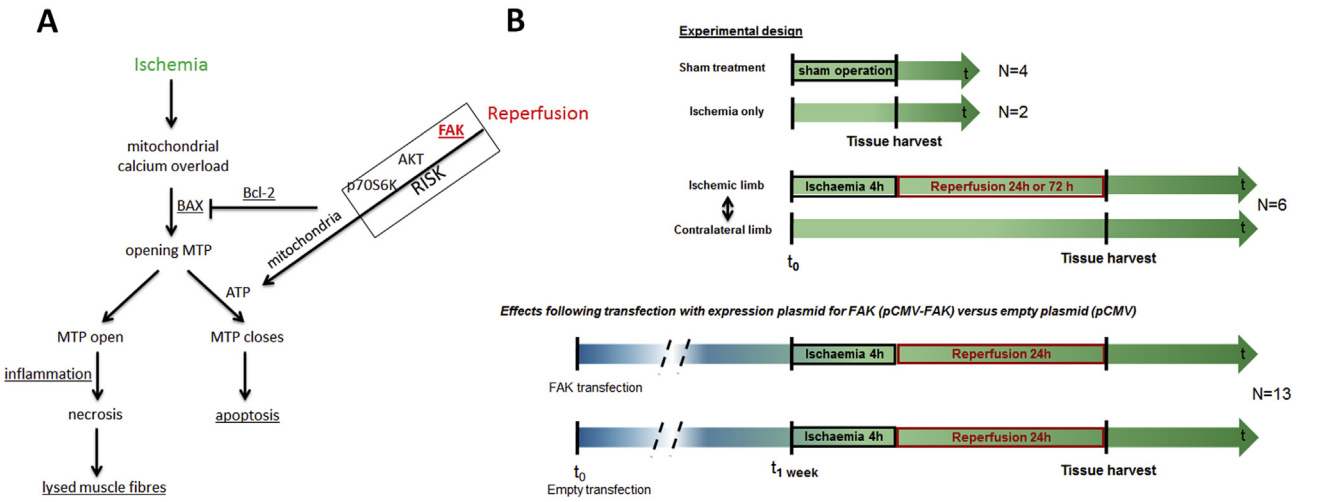

Figure 1. (A) Mechanisms of ischemia reperfusion damage and salvage and experimental protocol. RISK refers to a group of pro-survival protein kinases (FAK, AKT, and p70S6K) that confer muscle protection. During the first few minutes of reperfusion, a cellular increase in Ca^{2+} occurs which can be partially buffered by mitochondria. At a critical threshold, however, opening of permeability transition pores leads to cell death, either through energy dependent apoptosis, being fuelled by mitochondrial ATP production, or through necrosis. Fast-type muscle fibers have a lower mitochondrial content than slow-type muscle fibers and are more susceptible to IR injury. Endpoints, which were assessed using markers, are underlined and the factors being modulated experimentally are highlighted in color. (B) Experimental design. IR injury was evaluated following 4 hours of ischemia and 24 hours or 72 hours of reperfusion ($n = 6$ animals). Contralateral limbs served as paired controls, as represented by the double headed arrow. External controls were subjected to ischemia only ($n = 2$) or sham-treatment ($n = 4$). The effects following transfection with expression plasmid for FAK (pCMV-FAK) were compared with empty plasmid transfection (pCMV) ($n = 13$ animals). AKT = serine/threonine protein kinase AKT; BAX = apoptosis regulator BAX; Bcl-2 = apoptosis regulator Bcl-2; FAK = focal adhesion kinase; IR = ischemia reperfusion; MTP = mitochondria transition pore; p70S6K = ribosomal S6kinase; RISK = reperfusion induced salvage kinase. Note: The concept of ischemia and reperfusion injury was modified from refs 4,9,14.

mitochondria may explain why the degree of fiber injury has been found to vary between contractile muscle phenotypes.[6] Slow-type fibers generally have a higher mitochondria content than fast-type muscle. Mitochondria may act as a buffer against lethal calcium overload and fuel the energy dependent processes of apoptotic programming and self contained removal of cell debris. This may limit inflammation and lysis of damaged muscle fibers (Fig. 1A) and explain why fast-type muscle fibers are more prone to IR injury.

In the heart, anti-apoptotic signaling cascades, collectively referred to as the reperfusion injury salvage kinase (RISK) pathway, have gained much interest in acute myocardial infarction.[7–10] Among other effects, RISK leads to suppressed BAX action and prevents mitochondrial destruction.[4,5,9] So far, the therapeutic potential of this pathway has not been established in peripheral skeletal muscle. Modulation of focal adhesion kinase (FAK) offers one potential approach (Fig. 1A). FAK modulates integrin-based cell to cell matrix junctions that link the extracellular matrix to the cytoskeleton, which is key for maintaining tissue integrity, conveying tensile strength and transducing growth and survival signals. As upstream RISK regulator,[7,8,11] FAK reduces cell death by preventing mitochondrial perforation,[5] which has been shown to protect against IR injury in cardiac muscle.[7,8] Conversely, reduced FAK concentrations are considered hallmarks of impaired tissue oxygenation,[12] because FAK degradation is a natural consequence of tissue ischemia.[13] In skeletal muscle, FAK related effects of RISK activation and net mitochondrial biogenesis have been confirmed before.[14,15]

The aim of the present experimental pilot study was to determine whether FAK expression was modulated by IR and whether the enhancement of FAK expression had an impact on apoptosis and inflammation in IR-injured skeletal muscle.

METHODS

A reality driven rodent model of acute hind limb ischemia[16] was used to test whether enhanced FAK expression protected peripheral skeletal muscle from IR tissue injury. The hypothesis was that protective FAK effects should be most marked in muscle fibers with low mitochondrial content, because of their larger reserves towards an oxidative shift.[17] As in humans, rat fast-type glycolytic muscles (e.g., tibialis anterior and gastrocnemius muscles) have a lower mitochondrial content than slow-type oxidative muscles (i.e., soleus muscle).[18]

IR injury was compared across muscle phenotypes (i.e., gastrocnemius vs. soleus muscle) and the effect of enhanced FAK expression was evaluated. For gene transfer, the tibialis anterior was used as fast-type muscle because of its preferred *in vivo* accessibility as described before.[14,15] A paired design was adopted to differentiate local IR injury and protective effects from systemic reactions. Therefore, contralateral limbs served as intra-individual controls whereas external controls were subjected to ischemia only ($n = 2$) or sham treatment ($n = 4$). Fig. 1B summarizes the experiment.