INTERMITTENT PNEUMATIC COMPRESSION (IPC) INCREASES EXPRESSIONS OF eNOS mRNA AND PROTEIN IN LOCAL AND DISTANT SKELETAL MUSCLES

*Qi, W; +*Chen, L; *Tan, X; *Seaber, A V.; *Urbaniaiak, J R.
++Orthopaedic Microsurgery Laboratory, Duke University Medical Center, Durham, NC. PO Box 3093, DUMC, Durham, NC 27710, 919-684-5298, Fax: 919-681-8490, chen0006@mc.duke.edu

Introduction. Intermittent pneumatic compression (IPC) has been established as a method of clinically preventing deep vein thrombosis, but the mechanism is still unclear. Our earlier studies suggest that the application of IPC to rat hind limbs promotes vasodilation in the distant muscle, which is dose-dependently blocked by an inhibitor of NO synthase (NOS). We hypothesize that the vasodilation induced by IPC is, at least in part, via the release of NO. The direct evidence of NO mechanism, however, is still absent. Thus, we have measured the mRNA and protein expressions of three NOS isoforms --- neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) in local and distant skeletal muscles following the application of IPC.

Methods. Under anesthesia, 26 male rats weighing 250 - 300 g were divided into sham and IPC groups. The right anterior tibialis (AT) and cremaster muscle (CM) were removed from each rat as normal controls. After the wound was closed, a specially designed IPC device was applied on both legs of each rat. In the IPC, the pump of IPC provided a up to 55mmHg of pressure on both legs, with an inflation rate of 0.5 sec, an inflation duration of 5 sec, and a released duration of 25 sec. The same procedure was applied on the sham group except without IPC inflation. The duration of IPC application was 0.5, 1, 5 hrs, and 1 h of IPC plus 4 h of elapsed time, respectively.

Subsequently, the left AT (as a local muscle) and CM (as a distant muscle) were harvested, and RNA was immediately extracted using the TRIZol reagent. cDNA was synthesized using reverse transcriptase and Oligo-RT as a primer. PCR was performed by using cDNA as a template, and GAPDH as internal standard co-amplified with NO. All PCR product yields were measured during the exponential phase of amplification to detect differences in mRNA levels between samples. PCR band intensity was quantitated by densitometry. Relative mRNA expression for each specific NOS was calculated as a ratio of the densitometric value of NOS to the GAPDH, and then compared to normal value (Fig. 1). The muscles for Western blot was immediately frozen in liquid nitrogen and homogenized in lysis buffer. The homogenate was centrifuged and the protein concentration in the supernatants was determined by a BCA assay kit. 75µg of protein was separated on SDS-PAGE gels and then transferred onto nitrocellose membrane. The immunoblot was detected with isomorph-specific polyclonal anti-nNOS and eNOS antibodies and monoclonal anti-iNOS antibody, then incubated with HRP-conjugated secondary antibody and detected with an ECL detection kit. The relative level of NO protein was quantified by densitometry. The experiments were performed in triplicate. Ratios of gene and protein expressions were grouped according to treatment and the means compared for significance using ANOVA, with p<0.05 indicating significance.

Results: In the IPC group, nNOS mRNA expression in both AT and CM was unchanged or decreased after 0.5 and 1 h of IPC application, and then had a 1.5-fold increase at 5 hrs. An increase in normal in both muscles (Fig. 2). In contrast, eNOS mRNA was significantly up-regulated and reached a 2.7-fold increase from normal in both AT and CM at 5 hr of IPC application. Following 1 h of IPC plus 4 h of elapsed time, eNOS mRNA returned to near normal in both muscles (Fig. 2). Compared to sham groups, IPC application significantly (p<0.05) increased iNOS mRNA expression in CM (0.5 and 5 h) and nNOS in AT (1 h) and in CM (5 h). However, eNOS mRNA increased in both muscles at each time point, with a significant (p<0.05 to 0.01) difference, except CM following 5 h of IPC. Western blot showed that IPC application resulted in slight increased nNOS and significantly decreased NO in both muscles. However, eNOS was significantly up-regulated to 1.9- (1h) and 1.6-fold (5h) of normal in AT and 1.8- (1h) and 1.6-fold (5h) in CM. Compared to the sham group, the expression of eNOS protein was significantly up-regulated in both muscles after IPC application (Fig. 3). Similar to mRNA expression, eNOS returned to normal levels following 1 h of IPC and 4 h of elapsed time. Immunohistochemistry showed positive staining of eNOS in vascular endothelia of both AT and CM, which was deeper in the IPC-treated muscles than in normal and sham operation muscles.

Discussion: The data showed that eNOS mRNA and protein were significantly up-regulated in both local (AT) and distant (CM) muscles during IPC application, while nNOS slightly increased and iNOS significantly decreased. This NOS regulation by IPC was rapidly disappeared after stopping IPC application. The results confirm our hypothesis that the vasodilation of IPC in distant tissue is via NO mechanism, mainly due to the increase of eNOS instead of nNOS and iNOS. The IPC compression results in elevation of shear stress on the vascular wall in the compressed skeletal muscle, subsequently stimulating the endothelium, a biosensor of fluid dynamic shear force, by blood flow to synthesize NOS and release NO production. Released NO production promotes vasodilation in both local and distant skeletal muscles. Our results provide molecular evidence to prove NO mechanism of IPC application and support the clinical results by using IPC to prevent deep venous thrombosis.