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Far-infrared radiation acutely increases nitric oxide production by increasing Ca^{2+} mobilization and Ca^{2+}/calmodulin-dependent protein kinase II-mediated phosphorylation of endothelial nitric oxide synthase at serine 1179

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ABSTRACT

Repeated thermal therapy manifested by far-infrared (FIR) radiation improves vascular function in both patients and mouse model with coronary heart disease, but its underlying mechanism is not fully understood. Using FIR as a thermal therapy agent, we investigate the molecular mechanism of its effect on endothelial nitric oxide synthase (eNOS) activity and NO production. FIR increased the phosphorylation of eNOS at serine 1179 (eNOS-Ser^{1179}) in a time-dependent manner (up to 40 min of FIR radiation) in bovine aortic endothelial cells (BAEC) without alterations in eNOS expression. This increase was accompanied by increases in NO production and intracellular Ca^{2+} levels. Treatment with KN-93, a selective inhibitor of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and H-89, a protein kinase A inhibitor, inhibited FIR radiation-stimulated eNOS-Ser^{1179} phosphorylation. FIR radiation itself also increased the temperature of culture medium. As transient receptors potential vanilloid (TRPV) ion channels are known to be temperature-sensitive calcium channels, we explore whether TRPV channels mediate these observed effects. Reverse transcription-PCR assay revealed two TRPV isoforms in BAEC, TRPV2 and TRPV4. Although ruthenium red, a pan-TRPV inhibitor, completely reversed the observed effect of FIR radiation, a partial attenuation (~20%) was found in cells treated with Tranilast, TRPV2 inhibitor. However, ectopic expression of siRNA of TRPV2 showed no significant alteration in FIR radiation-stimulated eNOS-Ser^{1179} phosphorylation. This study suggests that FIR radiation increases NO production via increasing CaMKII-mediated eNOS-Ser^{1179} phosphorylation but TRPV channels may not be involved in this pathway. Our results may provide the molecular mechanism by which FIR radiation improves endothelial function.

1. Introduction

Far-infrared (FIR) radiation is an invisible electromagnetic wave with 3–1000 μm defined by the International Commission on Illumination [1]. FIR radiation transfers energy to the human body and manifests a wide variety of biological effects. These effects may be attributable that FIR radiation, via its specific range of frequency, activates the important molecules, such as water molecule in the human body, responsible for diverse biological effects. Several studies showed that FIR radiation has been reported for a long time to exert beneficial effects in cardiovascular systems [2]. For example, FIR irradiation decreased the vascular endothelial inflammation which was mediated by induction of heme oxygenase-1 [3]. Furthermore, repeated thermal therapy manifested by FIR also greatly improved impaired vascular endothelial function [4] and ventricular arrhythmias [5] in patients with chronic heart failure, and increased angiogenesis in a hindlimb ischemic mouse model. Later, increased eNOS expression was reported to be involved in one of the mechanisms underlying thermal therapy (thus FIR as well)-stimulated endothelial function and angiogenesis. However, a detailed molecular mechanism has not been elucidated.

Abbreviations: FIR, far-infrared; eNOS, endothelial nitric oxide synthase; eNOS-Ser^{1179}, eNOS at serine 1179; EC, endothelial cell(s); NO, nitric oxide; AMPK, AMP-activated protein kinase; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; PKA, protein kinase A; BAEC, bovine aortic EC; HUVEC, human umbilical vein EC; siRNA, small interference RNA; TRP, transient receptors potential; TRPV, TRP vanilloid; TRPM, TRP melastatin; TRPA, TRP ankyrin.

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Endothelial nitric oxide synthase (eNOS) is the major source of NO production in endothelial cells (EC). Dysregulation of eNOS is thought to contribute to the pathogenesis of cardiovascular diseases such as atherosclerosis and hypertension [6,7]. eNOS is mainly regulated at the level of phosphorylation [8]. Several specific sites of phosphorylation have been identified, among which eNOS-Ser^{1179} (bovine sequence) is the most studied. Phosphorylation of eNOS-Ser^{1179} increases NO production, mediated by several protein kinases, including Akt [9,10], AMP-activated protein kinase (AMPK) [11], Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) [12], protein kinase A (PKA) [13], and checkpoint kinase 1 [14]. The role of these protein kinases as signaling molecules for eNOS-Ser^{1179} phosphorylation is dependent on several stimuli including vascular endothelial growth factor (VEGF), bradykinin, shear stress, troglitazone, and UV irradiation [9–11,13,14]. In particular, it is well known that the increase in intracellular Ca^{2+} levels plays an important role in stimulating eNOS-Ser^{1179} phosphorylation and subsequent NO production through the reversible formation of the Ca^{2+}/calmodulin complex.

In this study, we investigate whether FIR increases NO production by activating a signaling axis in intracellular Ca^{2+} mobilization–CaMKII activation–eNOS-Ser^{1179} phosphorylation in BAEC.

2. Materials and methods

2.1. Materials

LY294002 (Akt inhibitor), Compound C (AMPK inhibitor), KN-93 (CaMKII inhibitor), BAPTA-AM (Ca^{2+} chelator), and ruthenium red (a pan-TRPV inhibitor) were purchased from Calbiochem (Darmstadt, Germany). Tranilast (an inhibitor of transient receptors potential vanilloid 2 (TRPV2) ion channels) and RN1734 (TRPV4 inhibitor) were purchased from A.G. Scientific (San Diego, CA) and Tocris Bioscience (Ellisville, MO), respectively. EGTA (extracellular Ca^{2+} chelator) and L-NAME (NOS inhibitor) were purchased from Sigma–Aldrich (St. Louis, MO). Antibodies against eNOS, p-eNOS-Ser^{1179}, p-CaMKII-Thr^{286}, and tubulin were purchased from Transduction Laboratories (Lexington, KY), Cell Signaling Technology (Boston, MA) and AbFrontier (Seoul, Korea), respectively. Minimal essential medium (MEM), Dulbecco's phosphate-buffered saline (DPBS), newborn calf serum (NCS), penicillin–streptomycin antibiotics, L-glutamine, and trypsin–EDTA solution obtained from Gibco-BRL (Gaithersburg, MD). All other chemicals were of the purest analytical grade.

2.2. Cell culture, FIR irradiation, and drug treatments

BAEC were isolated and maintained in MEM supplemented with 5% NCS at 37 °C under 5% CO_2 as described [15]. BAEC grown to 80% confluence were subjected to FIR radiation with wavelength between 6 and 20 μm using a ceramic FIR radiation generator, an S-O.T.M 9H FIR radiator (Saeik Medical Co Ltd, Bucheon, Korea). The radiator was set at a height of 30 cm above the bottom of culture plates, and the cells were exposed to FIR radiation at room temperature for the indicated times (0, 10, 20, 30, and 40 min). In some experiments, cells were pretreated for 1 h with 10 μM of Compound C, LY294002 or KN-93 in fresh MEM containing 0.5% NCS.

2.3. Transfection

Small interference RNA (siRNA) oligonucleotide designed against TRPV2 was synthesized as follows: 5′-ACU CAG UGC UGG AGA UCA UUU-3′ (Dharmacon Research Inc, Lafayette, CO). A non-specific siRNA oligonucleotide (Cat. No. D-001810-01) was also obtained for a control experiment. BAEC grown to 80% confluence in 60 mm culture dishes were transfected with 100 nM of each siRNA oligonucleotide using DharmaFECT (Dharmacon Research Inc.) according to manufacturer's instructions. After incubation for 5 h at 37 °C, DharmaFECT mixtures were washed out and the cells were further incubated in MEM containing 5% NCS for 24 h before FIR radiation.

2.4. Western blot analysis

For Western blot analysis, cells were treated without or with FIR radiation, washed with ice-cold DPBS and then lysed in lysis buffer, as previously described [9]. Protein concentrations were then determined using a BCA protein assay kit (Sigma–Aldrich). Equal quantities of protein (20 μg) were separated on SDS–PAGE under reducing conditions, after which they were electrophoretically transferred onto the nitrocellulose membranes. The blots were then probed with appropriate antibodies, followed by a corresponding secondary antibody, and finally developed using ECL reagents (Amersham, Buckinghamshire, UK).

2.5. Intracellular calcium measurement

Intracellular Ca^{2+} was detected by Fluo-4 AM (Invitrogen, Carlsbad, CA), an intracellular Ca^{2+} indicator, according to manufacturer's protocol. Briefly, BAEC grown on coverslips were treated without or with FIR radiation for the indicated times, and cells were then treated with 1 μM of Fluo-4 AM and fixed with 4% (wt/vol) paraformaldehyde. Images of intracellular Ca^{2+} were photographed using a confocal microscope (LSM5 Pascall, Carl ZEISS).

2.6. Reverse transcription-polymerase chain reaction (RT-PCR)

The total RNA from BAEC or human umbilical vein EC (HUVEC) were extracted using TRIzol reagent (Gibco-BRL, Gaithersberg, MD), as described previously [16]. cDNA was synthesized from total RNA using Superscript II reverse transcriptase and oligo-(dT)12–18 primer (Invitrogen), according to the manufacturer's instructions. The primer pairs for TRPV, TRP melastatin 8 (TRPM8), or TRP ankyrin 1 (TRPA1) were as follows: bovine TRPV1-F 5′-TGA CTC TGT GTC GGT CGA GT-3′ and bovine TRPV1-R 5′-GTG TTC CAG GTG GTC CAG TT-3′; bovine TRPV2-F 5′-TAC TAC ATG CGT GGC TTC CA-3′ and bovine TRPV2-R 5′-GAG ATG GCT TTC TGC AGC TT-3′; bovine TRPV3-F 5′-GAC ATC ACC TCG CAG GAC TC-3′ and bovine TRPV3-R 5′-GGC GAA CTT CTT CCA CTT CA-3′; bovine TRPV4-F 5′-CAA CTT GAA GGT GTG CGA TG-3′ and bovine TRPV4-R 5-TGG TTC CAG TGA GAC CAG TTC-3′; bovine TRPM8-F 5′-ATT CAC ATT TTC ACG GTC AGC-3′ and bovine TRPM8-R 5′-ACC TGG TCG TTG TTT TCC TG-3′; bovine TRPA1-F 5′-TCT CGT GGC TTT TGG ACT CT-3′ and bovine TRPA1-R 5′-TTT CAT GGG GGC AAA AGA TA-3′; bovine 18S rRNA-F 5′-GTT GGT GGA GCG ATT TGT CT-3′ and bovine 18S rRNA-R 5′-GGC CTC ACT AAA CCA TCC AA-3′; human TRPV1-F 5′-CTG TGC CGT TTC ATG TTT GT-3′ and human TRPV1-R 5′-TCT CCT GTG CGA TCT TGT TG-3′; human TRPV2-F 5′-TGT TGC CTA CCA TCA GCC TA-3′ and human TRPV2-R 5′-GTA GAT GCC TGT GTG CTG GA-3′; human TRPV3-F 5′-GGA AGA AGT TTG CCA AGC AC-3′ and human TRPV3-R 5′-GCA GGC GAG GTA CTC TTT GT-3′; human TRPV4-F 5′-TGT CCT GGT GAT CGT CTC AG-3′ and human TRPV4-R 5′-AAC AGG TCC AGG AGG AAG GT-3′; human TRPM8-F 5′-ATT CCG TTC GGT CAT CTA CG-3′ and human TRPM8-R 5′-GAA GGG GAA GGG GAT ATT GA-3′; human TRPA1-F 5′-GGA TCA GAA ATC CAC CAT CG-3′ and human TRPA1-R 5′-TGT GTT TTT GCC TTG ACT GC-3′; human 18S rRNA-F 5′-GCC GTT CTT AGT TGG TGG AG-3′ and human 18S rRNA-R 5′-GGG ACT TAA TCA ACG CAA GC-3′. PCR condition was one cycle at 94 °C for 5 min,

followed by 25 cycles at 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 1 min. All PCRs were performed in triplicates and detected by agarose gel electrophoresis.

2.7. Measurement of NO release

NO production was measured as nitrite (a stable metabolite of NO) concentration in cell culture supernatants as described [9]. BAEC were grown on 60 mm dish in culture media and incubated without or with FIR radiation for 30 min. After the end of incubation, 200 μl of culture media was carefully transferred into a 96-well plate, with the subsequent addition of 100 μl of Griess reagent (50 μl of 1% sulfanilamide containing 5% phosphoric acid and 50 μl of 0.1% N-(1-naphthyl) ethylenediamine). After color development at room temperature for 10 min, absorbance was measured on a microplate reader at a 520 nm wavelength.

2.8. Statistical analysis

All results are expressed as the means ± standard deviation (S.D.), with *n* indicating the number of experiments. Statistical significance was determined by a Student's *t*-test for two points. All differences were considered significant at a *P* value of <0.05.

3. Results

3.1. FIR radiation increases eNOS-Ser^{1179} phosphorylation and NO production in BAEC

Because NO production is mainly regulated by the phosphorylation of eNOS at serine 1179 site, we examined whether FIR radiation increases NO production by directly modulating this site. We found that FIR radiation significantly increased the phosphorylation of eNOS-Ser^{1179} in a time-dependent manner (Fig. 1A). Maximal increase in eNOS-Ser^{1179} phosphorylation was observed after 20 min treatment with FIR and this increase was maintained until 40 min of FIR radiation. Under these conditions, no alterations in eNOS expression were found (Fig. 1A). As expected, a significant increase in NO release was also found in cells treated with FIR for 30 min (Fig. 1B). Therefore, all subsequent experiments were accomplished with 30 min exposure of FIR, unless otherwise specifically stated.

3.2. CaMKII and PKA mediate FIR radiation-stimulated eNOS-Ser^{1179} phosphorylation

Because the phosphorylation of eNOS-Ser^{1179} is mediated by several protein kinases, including Akt, AMPK, PKA and CaMKII [9–13], we attempted to identify a kinase responsible for FIR radiation-stimulated eNOS-Ser^{1179}phosphorylation. As shown in Fig. 2, we found that H-89 and KN-93 inhibited eNOS-Ser^{1179} phosphorylation induced by FIR radiation, suggesting the involvement of PKA and CaMKII in the stimulatory effect of FIR radiation. However, treatment with inhibitors of other kinases, Akt and AMPK, which are also known to phosphorylate eNOS-Ser^{1179}, did not alter the observed effect of FIR radiation.

3.3. FIR radiation increases intracellular Ca^{2+} levels

Because CaMKII activity is modulated by intracellular Ca^{2+} levels, we hypothesized that FIR radiation increases CaMKII-mediated eNOS-Ser^{1179} phosphorylation via increasing intracellular Ca^{2+} levels. As shown in a Fig. 3A, confocal microscopy using Ca^{2+}-sensitive dye Fluo-4 AM revealed that FIR radiation significantly increases intracellular Ca^{2+} levels. Furthermore, BAPTA-AM (10 μM) completely abolished this increase (Fig. 3A) and subsequent FIR radiation-mediated increased phosphorylations of CaMKII-Thr^{286} and eNOS-Ser^{1179} (Fig. 3B). Moreover, the depletion of extracellular Ca^{2+} levels by EGTA (5 mM) also completely reversed a stimulatory effect of FIR radiation (Fig. 3C), suggesting a potential role for Ca^{2+} channel in FIR radiation-mediated signaling pathway.

3.4. TRPV does not mediate FIR-stimulated increase in eNOS-Ser^{1179} phosphorylation

It has been reported that mammalian cells possess at least six temperature-sensitive Ca^{2+} channels which activate Ca^{2+} entry in response to heat [17]. Because we also found that FIR radiation dramatically increases the temperature of culture media from 28 to 34.5 °C in a time-dependent manner (Fig. 4A), we examined which one, among reported thermo-sensitive Ca^{2+} channels, mediates the observed effect by FIR radiation. Under our experimental conditions, no alteration in cell viability was found (data not shown). Using RT-PCR analysis, we detected only two mRNA transcripts of thermo-sensitive, transient receptor potential vanilloid (TRPV) ion channels, TRPV2 and TRPV4, in BAEC (Fig. 4B), although TRPV1

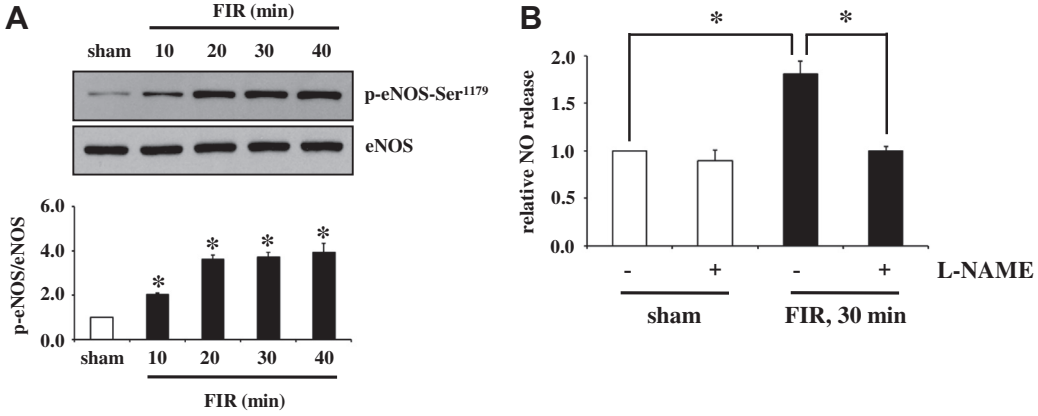

Fig. 1. FIR increases eNOS-Ser^{1179} phosphorylation and NO production in BAEC. BAEC were exposed to FIR for various times (0, 10, 20, 30, or 40 min) at room temperature. Control cells (sham) were not exposed. After radiation, cells were harvested with lysis buffer as mentioned in Section 2. For Western blot analysis, cell proteins separated on SDS–polyacrylamide gel were electrophoretically transferred onto nitrocellulose membranes. Proteins transferred on membranes were immunoblotted with antibodies specific for eNOS phosphorylated at Ser 1179 (p-eNOS-Ser^{1179}) and total eNOS (A). After pretreatment without or with L-NAME (1 mM), cells were exposed to FIR for 30 min, and NO release was measured by the Griess method (B). Each bar shows the mean p-eNOS-Ser^{1179} (A) and NO production (B) (after normalization to total cellular protein as fold increases above controls ± S.D.) and represents at least four experiments. Differences were statistically significant at $^{*}P < 0.05$.

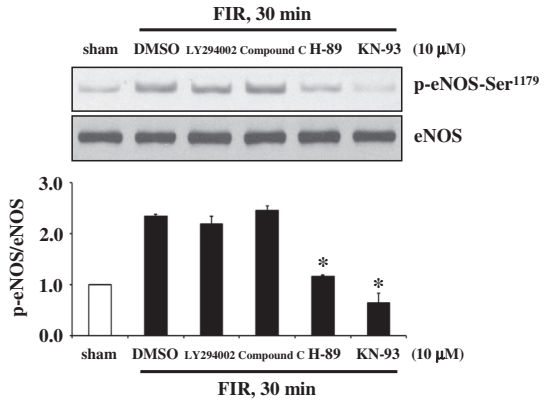

Fig. 2. CaMKII or PKA reverses FIR radiation-mediated increase in eNOS-Ser^{1179} phosphorylation. After pretreatment with LY294002 (Akt inhibitor), Compound C (AMPK inhibitor), KN-93 (CaMKII inhibitor), or H-89 (PKA inhibitor) for 1 h, the cells were exposed to FIR for 30 min. For DMSO control, cells were pretreated without drugs but exposed to FIR. Sham control cells were not exposed to FIR. The levels of p-eNOS-Ser^{1179} and eNOS were measured using corresponding antibodies as described in the legends of Fig. 1. Bar shows the mean fold alterations below the DMSO controls (±S.D.) as described in the legend of Fig. 1. Differences were statistically significant at $^{*}P < 0.05$ ($n = 4$).

mRNA was also detected in HUVEC In addition to these two mRNA transcripts. We also found that ruthenium red, known as a pan-TRPV inhibitor, significantly inhibits increase in eNOS-Ser^{1179} phosphorylation induced by FIR radiation (Fig. 4C), suggesting that either TRPV1 or TRPV2 is likely to be involved in this signaling pathway in BAEC. Next, we investigated which isoform indeed plays role in mediating FIR radiation-stimulated eNOS-Ser^{1179} phosphorylation, and found that Tranilast, an inhibitor of TRPV2, partially (~20%) inhibits eNOS-Ser^{1179} phosphorylation, but RN1734, TRPV4 inhibitor, did not (Fig. 4D). However, although ectopic transfection with siRNA of TRPV2 was successful (Fig. 4E), this ectopic expression did not alter FIR radiation-stimulated increase in eNOS-Ser^{1179} phosphorylation (Fig. 4F), suggesting no clear evidence for involvement of TRPV2 in this signaling pathway in BAEC.

4. Discussion

FIR therapy has been reported to reduce several cardiovascular risk factors including high blood pressure. Although increased eNOS expression and NO production are considered to mediate the improvement of impaired vascular endothelial function by FIR radiation, its detailed mechanism has not been fully elucidated. In this study, we demonstrate that intracellular Ca^{2+} mobilization and CaMKII mediate the acute effect of FIR radiation on increased NO production through eNOS-Ser^{1179} phosphorylation. However, our data suggest no evidence for the involvement of thermo-sensitive TRPV Ca^{2+} channels in FIR radiation-mediated observed effect.

Several studies have shown that CaMKII is involved in NO production through Ca^{2+}-dependent eNOS-Ser^{1179} phosphorylation. In this study, we found that FIR radiation acutely increases CaMKII activity, as evidenced by increased CaMKII-Thr^{286} phosphorylation, which is completely inhibited by calcium scavengers, both BAMTA-AM and EGTA. From these results, we hypothesized that FIR radiation activates Ca^{2+} channels at level of cell membrane, thus increasing intracellular Ca^{2+} levels and subsequently a signaling

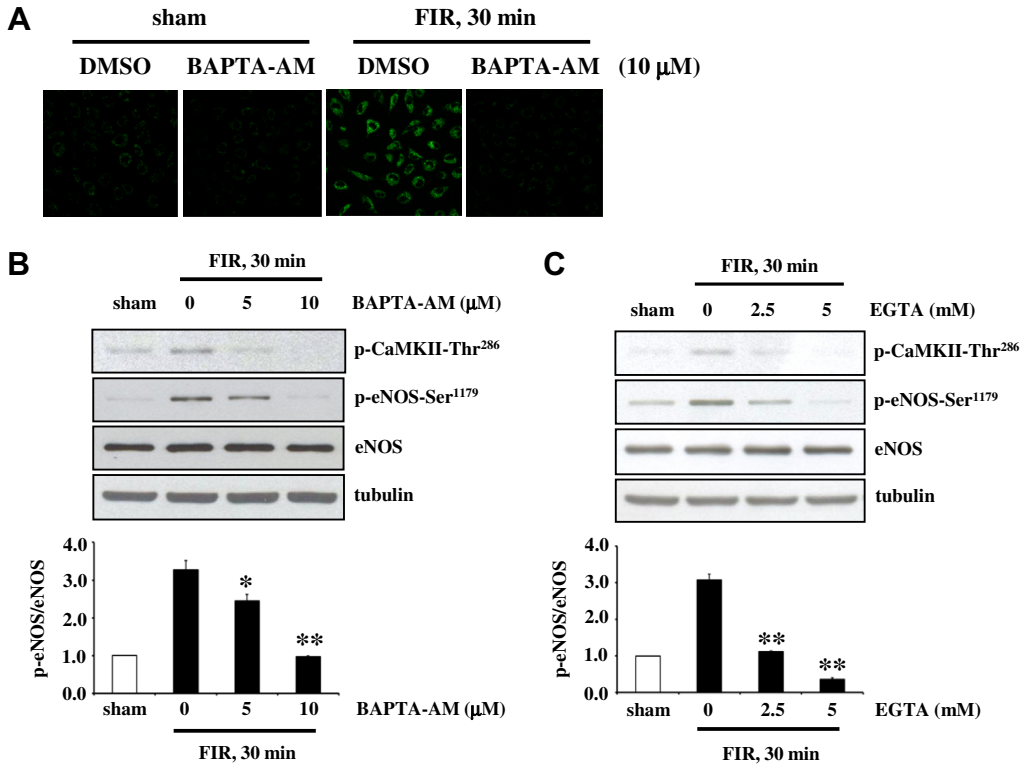

Fig. 3. FIR radiation increases intracellular Ca^{2+} levels. BAEC were exposed to FIR for 30 min in culture media containing 1 μM Fluo-4 AM after pretreatment without or with 10 μM BATA-AM. Two types of control cells (DMSO or sham) were treated as described in the legends of Fig. 2. Ca^{2+} levels were visualized by confocal microscopy as described in Section 2 (A). In separate experiments, cells were also pretreated with various doses of either BAPTA-AM (0, 5 or 10 μM) (B) or EGTA (0, 2.5 or 5 mM) (C) and exposed to FIR for 30 min. After radiation, cells were harvested with lysis buffer as mentioned in Section 2. Levels of p-eNOS-Ser^{1179}, total eNOS and CaMKII phosphorylated at Thr286 (p-CaMKII-Thr^{286}) were measured using corresponding antibodies as described in the legends of Fig. 1. Bar shows the mean fold alterations below the DMSO controls (±S.D.) as described in the legend of Fig. 1. Differences were statistically significant at $^{*}P < 0.05$ or $^{**}P < 0.01$ ($n = 4$).

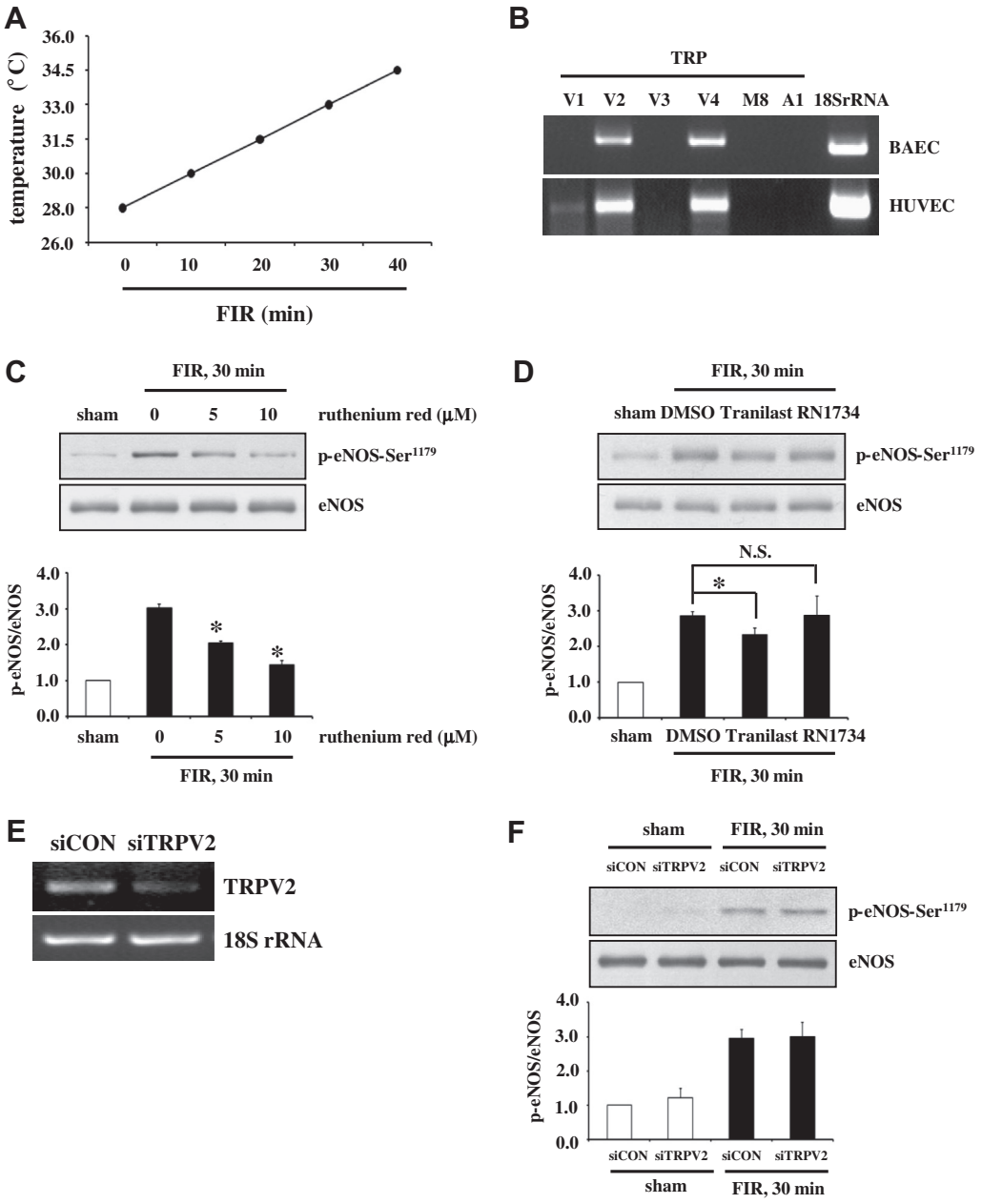

Fig. 4. TRPV does not mediate FIR-stimulated increase in eNOS-Ser^{1179} phosphorylation. Cells were exposed to FIR as described in the legends of Fig. 1, and temperature of culture media was measured directly by the thermometer (A). The total RNA from BAEC and HUVEC was extracted using TRIzol reagent. mRNA transcript levels of each thermosensitive-TRP channels were measured by RT-PCR assay as described in Section 2 (B). BAEC were pretreated with various doses of ruthenium red (0, 5 or 10 μM) (C), Tranilast as TRPV2 inhibitor (75 μM), or RN1734 as TRPV4 inhibitor (40 μM) (D). After pretreatment or transfection, cells were exposed to FIR for 30 min. In some experiments, BAEC were also ectopically transfected for 24 h with 100 nM siRNA specific for TRPV2 gene (siTRPV2) or non-specific siRNA (siCON). After transfection, cells were also exposed to FIR for 30 min (F). Two types of control cells (DMSO or sham) were treated as described in the legends of Fig. 2. Cell lysates after treatments were prepared and the levels of p-eNOS-Ser^{1179} and eNOS were analyzed using corresponding antibodies as described in Fig. 1. Bar shows the mean fold alterations below the DMSO controls (±S.D.) as described in the legend of Fig. 1. Differences were statistically significant at $^{*}P < 0.05$ or $^{**}P < 0.01$ ($n = 4$). N.S., not significant.

axis in CaMKII phsophorylation–eNOS-Ser^{1179} phosphorylation–NO production in BAEC. In this regard, it was previously reported that the pulsed IR radiation evokes intracellular Ca^{2+} transients, resulting in excitability in both excitable and non-excitable cells such as cardiomyocytes, rat pheochromocytoma PC12 cells, and HeLa cells [18–21]. However, our findings using FIR radiation differed from those of the previous studies; in our study, removal of extracellular Ca^{2+} by EGTA attenuated completely FIR radiation-mediated NO signaling pathway in BAEC (Fig. 3C), suggesting a role of membrane Ca^{2+}channels in FIR effect. The previous studies using pulsed IR, however, failed to find extracellular Ca^{2+} effects; they highlighted a role for intracellular Ca^{2+} storage, such as endoplasmic reticulum and mitochondria, in IR-derived various cellular functions. Different cell culture and experimental conditions may explain these apparently incompatible observations, although more detailed studies are needed to clarify this issue. In the present study, we used BAEC and FIR radiation ranging 6–20 μm wavelength, while the previous studies used different types of cells such as cardiac, neuronal and HeLa cells, and near-IR radiation with shorter wavelength of ~0.8 to ~1.8 μm.

Because TRPV are known to be thermo-sensitive, Ca^{2+} channels working at the level of membrane in a variety of cells, it is reasonable to think that TRPV mediate FIR radiation-induced increased Ca^{2+} levels in BAEC. Several previous studies addressed

that FIR radiation manifests a wide variety of biological effects at least in part via heat transfer. In fact, under our conditions, we also found that FIR radiation significantly increases temperature of culture media from 28 to 34.5 °C, suggesting a thermal effect of FIR radiation in the observed effect. However, we were unable to find a clear evidence for the involvement of TRPV isoforms used in this study in the observed effects of FIR radiation in BAEC. Recently, using experimental mice, capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide), the major pungent ingredient in hot pepper, was reported to improve vasorelaxation and prevent hypertension in part by increasing the phosphorylation of eNOS-Ser^{1177} (which is comparable to eNOS-Ser^{1179} in BAEC) and NO release in EC [22]. Furthermore, the authors of the previous study also proposed that TRPV1 mediates capsaicin-induced increases in eNOS-Ser^{1177} phosphorylation and NO production. Although intracellular Ca^{2+} levels were not measured in this previous study, they reported that PKA activity is clearly involved in the signaling axis in TRPV1 activation–eNOS-Ser^{1177} phosphorylation–NO production. In this regard, we also found that PKA in addition to CaMKII may be involved in the stimulatory effects of FIR radiation in NO release in BAEC. Unlike mouse EC, however, our study shows that TRPV1 is unlikely to mediate the observed effects of FIR radiation because TRPV1 is not detected (Fig. 4B) and capsaicin does not increase eNOS-Ser^{1179} phosphorylation (data not shown) in BAEC. Although these different cell-specific effects of TRPV1 in NO signaling pathway are interesting, further study is needed to clarify this issue. In this study, we found that TRPV2 inhibitor partially (~20%) attenuates FIR radiation-mediated increase in eNOS-Ser^{1179} phosphorylation. However, it is unlikely that TRPV2 also mediates this signaling pathway because treatment with siRNA of TRPV2, which is more likely specific relative to use of an inhibitor, did not affect eNOS-Ser^{1179} phosphorylation by FIR radiation. It is noted that TRPV2 is activated by much higher temperature (52 °C) than that in our study, which is reported to be tightly regulated in various types of cells. Nonetheless, because either ruthenium red, general inhibitor for TRP Ca^{2+} channels, or EGTA, an impermeable and extracellular Ca^{2+} chelator, completely reversed the stimulatory effect of FIR radiation on increased eNOS-Ser^{1179} phosphorylation, it is likely that there exists unknown temperature-sensitive, Ca^{2+} channel mediating FIR radiation-stimulated NO production in BAEC, which may be different from known TRPV isoforms.

In summary, this study is the first to show that FIR radiation increases eNOS-Ser^{1179} phosphorylation and NO production in BAEC through intracellular Ca^{2+} mobilization and CaMKII activation. PKA is also involved, but we are unable to find a clear evidence for the involvement of well-known TRPV isoforms in this pathway. Because FIR radiation has valuable features such as a quick and controllable turning the effects on and off compared with conventional drug treatment, our study may provide a therapeutic potential in NO-related vascular dysfunction.

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References

[1] International Commission on Non-Ionizing Radiation Protection, ICNIRP statement on far infrared radiation exposure, Health Phys. 91 (2006) 630–645.
[2] R. Beever, Far-infrared saunas for treatment of cardiovascular risk factors: summary of published evidence, Can. Fam. Physician 55 (2009) 691–696.
[3] C.C. Lin, X.M. Liu, K. Peyton, H. Wang, W.C. Yang, S.J. Lin, W. Durante, Far infrared therapy inhibits vascular endothelial inflammation via the induction of heme oxygenase-1, Arterioscler. Thromb. Vasc. Biol. 28 (2008) 739–745.
[4] T. Kihara, S. Biro, M. Imamura, S. Yoshifuku, K. Takasaki, Y. Ikeda, Y. Otuji, S. Minagoe, Y. Toyama, C. Tei, Repeated sauna treatment improves vascular endothelial and cardiac function in patients with chronic heart failure, J. Am. Coll. Cardiol. 39 (2002) 754–759.
[5] T. Kihara, S. Biro, Y. Ikeda, T. Fukudome, T. Shinsato, A. Masuda, M. Miyata, S. Hamasaki, Y. Otsuji, S. Minagoe, S. Akiba, C. Tei, Effects of repeated sauna treatment on ventricular arrhythmias in patients with chronic heart failure, Circ. J. 68 (2004) 1146–1151.
[6] B.S. Oemar, M.R. Tschudi, N. Godoy, V. Brovkovich, T. Malinski, T.F. Luscher, Reduced endothelial nitric oxide synthase expression and production in human atherosclerosis, Circulation 97 (1998) 2494–2498.
[7] H. Duplain, R. Burcelin, C. Sartori, S. Cook, M. Egli, M. Lepori, P. Vollenweider, T. Pedrazzini, P. Nicod, B. Thorens, U. Scherrer, Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase, Circulation 104 (2001) 342–345.
[8] P.F. Mount, B.E. Kemp, D.A. Power, Regulation of endothelial and myocardial NO synthesis by multi-site eNOS phosphorylation, J. Mol. Cell. Cardiol. 42 (2007) 271–279.
[9] D.H. Cho, Y.J. Choi, S.A. Jo, I. Jo, Nitric oxide production and regulation of endothelial nitric-oxide synthase phosphorylation by prolonged treatment with troglitazone, J. Biol. Chem. 279 (2004) 2499–2506.
[10] D. Fulton, J.P. Gratton, T.J. McCabe, J. Fontana, Y. Fujio, K. Walsh, T.F. Franke, A. Papapetropoulos, W.C. Sessa, Regulation of endothelium-derived nitric oxide production by the protein kinase Akt, Nature 399 (1999) 597–601.
[11] Y. Zhang, T.S. Lee, E.M. Kolb, K. Sun, X. Lu, F.M. Sladek, G.S. Kassab, T. Garland Jr., J.Y. Shyy, AMP-activated protein kinase is involved in endothelial NO synthase activation in response to shear stress, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 1281–1287.
[12] J.C. Schneider, D. El Kebir, C. Chéreau, S. Lanone, X.L. Huang, A.S. De Buys Roessingh, J.C. Mercier, J. Dall'Ava-Santucci, A.T. Dinh-Xuan, Involvement of Ca2+/calmodulin-dependent protein kinase II in endothelial NO production and endothelium-dependent relaxation, Am. J. Physiol. Heart Circ. Physiol. 284 (2003) H2311–H2319.
[13] S.W. Bae, H.S. Kim, Y.N. Cha, Y.S. Park, S.A. Jo, I. Jo, Rapid increase in endothelial nitric oxide production by bradykinin is mediated by protein kinase A signaling pathway, Biochem. Biophys. Res. Commun. 306 (2003) 981–987.
[14] J.H. Park, W.S. Kim, J.Y. Kim, M.H. Park, J.H. Nam, C.W. Yun, Y.G. Kwon, I. Jo, Chk1 and Hsp90 cooperatively regulate phosphorylation of endothelial nitric oxide synthase at serine 1179, Free Radic. Biol. Med. 51 (2011) 2217–2226.
[15] H.P. Kim, J.Y. Lee, J.K. Jeong, S.W. Bae, H.K. Lee, I. Jo, Nongenomic stimulation of nitric oxide release by estrogen is mediated by estrogen receptor localized in caveolae, Biochem. Biophys. Res. Commun. 263 (1999) 257–262.
[16] J. Min, Y.M. Jin, J.S. Moon, M.S. Sung, S.A. Jo, I. Jo, Hypoxia-induced endothelial NO synthase gene transcriptional activation is mediated through the tax-responsive element in endothelial cells, Hypertension 47 (2006) 1189–1196.
[17] L. Vay, C. Gu, P.A. McNaughton, Current perspectives on the modulation of thermo-TRP channels: new advances and therapeutic implications, Expert Rev. Clin. Pharmacol. 3 (2010) 687–704.
[18] G.M. Dittami, S.M. Rajguru, R.A. Lasher, R.W. Hitchcock, R.D. Rabbitt, Intracellular calcium transients evoked by pulsed infrared radiation in neonatal cardiomyocytes, J. Physiol. 589 (2011) 1295–1306.
[19] N.I. Smith, S. Iwanaga, T. Beppu, K. Fujita, O. Nakamura, S. Kawata, Photostimulation of two types of Ca2+ waves in rat pheochromocytoma PC12 cells by ultrashort pulsed near-infrared laser irradiation, Laser Phys. Lett. 3 (2006) 154–161.
[20] V. Tseeb, M. Suzuki, K.K. Oyama, K. Iwai, S. Ishiwata, Highly thermosensitive Ca dynamics in a HeLa cell through IP(3) receptors, HFSP J. 3 (2009) 117–123.
[21] J. Ando, N.I. Smith, K. Fujita, S. Kawata, Photogeneration of membrane potential hyperpolarization and depolarization in non-excitable cells, Eur. Biophys. J. 38 (2009) 255–262.
[22] D. Yang, Z. Luo, S. Ma, W.T. Wong, L. Ma, J. Zhong, H. He, Z. Zhao, T. Cao, Z. Yan, D. Liu, W.J. Arendshorst, Y. Huang, M. Tepel, Z. Zhu, Activation of TRPV1 by dietary capsaicin improves endothelium-dependent vasorelaxation and prevents hypertension, Cell Metab. 12 (2010) 130–141.