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·基础研究·

Trichloroethylene Alters Balance of Nitric Oxide and Superoxide Anion on Confluent Endothelial Cell Culture

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Abstract: 【Objective】 Trichloroetheylene (TRI) is an environmental pollutant that has been linked to congenital heart defects. Our earlier report showed that TRI inhibited stimulated nitric oxide (NO) production and increased stimulated endothelial nitric oxide synthase (eNOS)- dependent superoxide anion (O_2^-) production by decreasing heat shock protein 90(hsp90) associated with eNOS and limited vascular endothelial growth factor-stimulated proliferation on proliferating endothelial cells (EC). The effects of TRI on confluent EC remained unknown. The aim of this study was to test the effects of TRI on confluent EC. 【Method】 Confluent EC were pretreated with TRI (5 μmol/L) and then stimulated with the calcium ionophore, A23187 (5 μmol/L), to determine changes in EC and eNOS functions with respect to NO and O_2^- generation. Immunoblots of eNOS, phosphorylation of eNOS (P-eNOS) at serine 1179 (S1179) and the levels of associated hsp90 with eNOS were used to define the activation state of eNOS. 【Results】 TRI decreased A23187-stimulated nitrite + nitrate production from (.16 ± 0.15) nmol · mg^{-1} to (0.91 ± 0.3) nmol · mg^{-1} ($P < 0.05$). In controls, *L*-nitroargininemethylester (*L*-NAME) increased A23187-stimulated O_2^- production from (0.015 ± 0.007) nmol / (min · mg) to (0.044 ± 0.008) nmol / (min · mg) ($P < 0.05$). In TRI-treated cultures, however, *L*-NAME decreased A23187-stimulated O_2^- production from (0.057 ± 0.022) nmol / (min · mg) to (0.039 ± 0.005) nmol / (min · mg) ($P < 0.05$) and oxypurinol decreased A23187-stimulated O_2^- production from(0.05 ± 0.022) nmol/ (min · mg) to 0.034 ± 0.012 nmol/ (min · mg) ($P < 0.05$). TRI has no effect on eNOS, p-eNOS, and hsp90 expression but decreased hsp90 association with eNOS. 【Conclusion】 These data show that TRI not only alters hsp90 interaction with eNOS and shifts eNOS from · NO to O_2^- generation, but also activates a second oxidative enzyme, xanthine oxidase, to increase O_2^- generation in confluent EC. Such changes in endothelial functions may contribute to many cardiovascular diseases.

Key words: heat shock protein 90; nitric oxide synthase; nitric oxide; superoxide; endothelial cell
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Trichloroethylene 改变融合期内皮细胞一氧化氮和氧自由基的平衡

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摘 要: 【目的】 Trichloroethylene (TRI) 是一种与先天性心脏病发生有关的环境污染物,我们最近报道在分

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裂期的内皮细胞（EC）中，TRI 可以通过减少热休克蛋白 90（hsp90）与内皮一氧化氮合酶（eNOS）结合，从而抑制刺激状态下一氧化氮（NO）产生和增加刺激状态下 eNOS 依赖的超氧阴离子自由基（O_2^-）产生和抑制血管内皮生长因子刺激的内皮细胞分裂。但 TRI 在融合期 EC 中的影响仍未清楚。本实验目的是探讨 TRI 对融合期 EC 的影响。【方法】 TRI 5 μmol/L **预处理融合期** EC，**用钙离子导体** A23187 5 μmol/L **刺激，然后，检测** NO **与** O_2^- **的产生以了解** EC **和** eNOS **的功能情况。用免疫印迹方法检查** eNOS，**位点于丝氨酸** 1179 **的磷酸化** eNOS，**与** eNOS **结合的** hsp90 **水平来决定** eNOS **的激活状态。【结果】** TRI **减少** A23187 **刺激状态下亚硝酸盐和硝酸盐的产生从**（1.16 ± 0.15）nmol·mg^{-1} **到**（0.91 ± 0.3）nmol·mg^{-1}（$P < 0.05$）；**在对照情况下，***L*-NAME **增加** A23187 **刺激状态下的** O_2^- **产生从**（0.015 ± 0.007）nmol/（min·mg）**到**（0.044 ± 0.008）nmol/（min · mg）（$P < 0.05$）；**在** TRI **处理的培养中，***L*-NAME **减少** A23187 **刺激状态下的** O_2^- **产生从**（0.057 ± 0.022）nmol/（min · mg）**到**（0.039 ± 0.005）nmol/（min · mg）（$P < 0.05$），**并且** oxypurinol **减少** A23187 **刺激状态下的** O_2^- **产生从**（0.057 ± 0.022）nmol/（min · mg）**到**（0.034 ± 0.012）nmol/（min · mg）（$P < 0.05$）；TRI **对** eNOS, p-eNOS **和** hsp90 **的表达无影响，但减少** hsp90 **与** eNOS **的结合。【结论】这些数据表明，在融合期** EC **中，**TRI **不但改变** hsp90 **与** eNOS **结合，使** eNOS **从产生** NO **变为产生** O_2^-，**而且激活第二氧化酶 - 黄嘌呤氧化酶，而增加** O_2^- **产生。这些内皮功能的改变可能导致许多心血管疾病的发生。**

关键词：热休克蛋白 90；一氧化氮合酶；一氧化氮；氧自由基；内皮细胞

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Trichloroethylene (TRI) is an industrial solvent that is commonly used as a degreaser and cleaning agent. A lot of evidences suggest that fetal exposure to TRI induces congenital heart diseases (CHD) [1]. However, the cellular mechanisms of TRI-induced cardiac teratogenicity are still unclear. Boyer used an *in vitro* chick-AV canal culture model to determine the effects of TRI on the development of cardiac valves and septa[2]. They found that TRI affected several elements of epithelial- mesenchymal cell transformation and blocked endothelial cell-to-cell separation processes that are associated with endothelial activation. Nitric oxide (NO) has been demonstrated to play an important role in new blood vessel growth and many cardiovascular diseases[3]. Endothelial nitric oxide synthase (eNOS) is the major source of NO in vascular endothelial cells and cardiomyocytes in response to a variety of agonists and mechanical stimuli. A number of investigations indicate that eNOS plays an important role in heart development and angiogenesis[4, 5]. Heat shock protein 90 (hsp90) is one of protein in the chaperone family that interacts with eNOS and governs its function[6-9]. Our recent report showed that TRI altered hsp90 interactions with eNOS and shifted eNOS production from NO to superoxide (O_2^-). These data provide a mechanistic basis for TRI impairing vascular endothelial growth factor (VEGF)-stimulate endothelial proliferation in proliferation endothelial cells (EC) and suggest that such changes may play an important role in development of CHD[10]. Although growing evidence indicates that TRI increases risk of CHD, few studies examine the effects of TRI on confluent EC function which has greater importance to cardiovascular disease related to hypertension, coronary artery disease, atherosclerosis or even cardioprotection during cardiac surgery. As proliferating EC are different from confluent EC with respect NO and O_2^- generation, the effects and mechanisms of TRI on EC functions may also be different[11, 12]. In this study, we examine the effects of TRI on confluent EC NO and O_2^- generation and hsp90 interaction with eNOS to determine how TRI alters endothelial and eNOS functions on confluent EC.

1　Materials and Methods

1.1　Materials

L^{ω}-Nitroargininemethylester (*L*-NAME), N^{G}-methyl-*L*-arginine monoacetate (*L*-NMA), Oxypurinol, dimethyl sulfoxide (DMSO), antibiotics/mycotics,

trypsin-EDTA, Hank's balanced salts solution (HBSS), *L*-arginine, sodium nitrite, ferricytochrome c, superoxide dismutase (SOD), NaF, sodium deoxycholate, sodium dodecyl sulfate (SDS), 4-(2-aminoethyl benzene) sulfonyl fluoride hydrochoride, sodium orthovanadate, leupeptin, pepstatin A, aprotinin, protein A-Sepharose were from Sigma Chemical Company (St. Louis, MO). RPMI 1640 was from Invitrogen Corporation (Grand Island, NY). Tris-HCl was from Baker (Phillipsburg, NJ). Triton X-100 was from Lab Chem (Pittsburg, PA). A23187, a calcium ionophore, was from Calbiochem (San Diego, CA). Laemmli buffer, polyacrylamide, nitrocellulose membranes were from BioRad (Hercules, CA). Fetal bovine serum (FBS) was from HyClone (Logan, UT). ECL reagents were from Amersham Pharmacia Biotech (Piscataway, NJ). X-OMAT film was from Kodak (Rochester, NY). H32 antibody was from BioMol (Plymouth Meeting, PA). Anti-eNOS was from Zymed Laboratories (San Francisco, CA) (Catalog# 33-4600, 9D10). Anti-hsp90 (H38220) was from Transduction Laboratories (Franklin, NJ). Anti-P-eNOS (Ser-1177) was from Cell Signaling Technology (Beverly, MA). Trichloroethylene and Vanadium (Ⅲ) chloride were from Fluka-Aldrich Chemical Company (Milwaukee, WI).

1.2 Methods

1.2.1 Endothelial Cell Culture Bovine coronary endothelial cells (BCEC) were provided by William B. Campbell, Medical College of Wisconsin (Milwaukee, WI). BCEC were cultured in RPMI 1640 media containing φ = 20% fetal bovine serum, penicillin, streptomycin and amphotericin B as before[6]. BCEC were passaged with trypsin-EDTA and used for experiments between passages 6-8. When BCEC grow confluent (95% confluent, confirmed by microscope), then was used.

1.2.2 Preparation of TRI Working Stock Solutions Stock solutions of TRI were made by adding TRI (neat) directly to a varying volume of ethanol (95%) so as to be able to add constant volume of the working stock TRI solutions to the culture media (final concentration of ethanol, 15 mmol/L) to achieve the desired final concentration (5 μmol/L). An equal volume of the ethanol vehicle was added to all paired controls.

1.2.3 Determination of Nitrite + Nitrate To assess changes in nitrite + nitrate production in the presence and absence of TRI, TRI (5 μmol/L, final concentration) or ethanol vehicle was added to confluent BCEC in 6-well dishes overnight. The next day, *L*-NMA (1 mmol/L, final concentration) was added in parallel at time 0. At 30 min, the cultures were washed three times with HBSS. After the third wash, the test-groups were incubated in 1 mL HBSS containing A23187 (5 μmol/L, final concentration) and *L*-arginine (25 μmol/L, final concentration) for 30 min. The HBSS was removed and nitrite + nitrate was quantified using a Sievers NOA analyzer as described[13].

1.2.4 Determination of Superoxide Anion TRI (5 μmol/L, final concentration) was added to confluent BCEC in three of five 6-well plates. The next day, *L*-NAME (1 mmol/L, final concentration) was added to one nonTRI-treated and one TRI-treated confluent BCEC cultures and oxypurinol was added to one TRI-treated confluent BCEC cultures at time 0. At 30 min, the test groups were washed three times with HBSS. After the final HBSS wash, the five test groups in 6-well dishes (Control, *L*-NAME, TRI, TRI + L-NAME and TRI + oxypurinol) were incubated in 1 mL of HBSS containing ferricytochrome c (c_t = 50 μmol/L, final concentration) and A23187 (5 μmol/L, final concentration) with and without *L*-NAME (1 mmol/L, final concentration) or oxypurinol (100 μmol/L, final concentration) for 30 min. Superoxide anion production was calculated from the absorbance of ferricytochrome c at 550 nm and the molar extinction coefficient (ε = 21, 000 $M^{-1} \cdot cm^{-1}$) as described [6]. SOD-inhibitable O_2^- data were calculated from the release of O_2^- from independent wells incubated with HBSS containing ferricytochrome c with and without

SOD (1 000 units/mL, final concentration).

1.2.5 Western Blots for eNOS, hsp90 and Phosphorylation of eNOS (S1179) Western blot analysis was used to determine if TRI altered endothelial cell expression of eNOS or hsp90 or altered the phosphorylation of eNOS at S1179 in control and TRI-treated cultures. Briefly, confluent BCEC cultures in 60-mm dishes were pretreated with TRI (5 μmol/L, final concentration) overnight. The cultures were washed three times with HBSS and then incubated with 10 μmol/L *L*-arginine supplemented HBSS with and without A23187 (5 μmol/L, final concentration). After incubation for 10 min at 37 ℃, the HBSS solutions were removed by aspiration, and cell proteins were harvested in 500 μL of modified RIPA buffer, as described [6]. Western blot was performed as before[10]. The proteins were transferred to nitrocellulose membranes and blotted with anti-P-eNOS (Ser-1177), anti-eNOS (9D10) and anti-hsp90 overnight at 4 ℃. Bands were visualized using horseradish peroxidase (HRP)-linked secondary antibodies and ECL reagents[6].

To determine if TRI altered protein interactions between hsp90 and eNOS, the experiment described above was repeated on confluent BCEC cultures in 100 mm dishes to increase the amount of protein in cell lysates for immunoprecipitation of eNOS. After incubation with TRI overnight, the four test groups (Control, TRI, Control + A23187, and TRI + A23187) were washed three times with 6 mL of HBSS and then incubated at 37 ℃ in a tissue culture incubator in 6 ml of HBSS containing *L*-arginine (10 μmol/L) with and without A23187 (5 μmol/L). After 10 min, the HBSS was removed and the cells were lysed in modified RIPA buffer and samples processed as previously described[6]. Immunoprecipitation of eNOS was performed as before[10]. The membrane was blocked with 5% nonfat milk in TBS-Tween 20 (0.1%) and immunoblotted for eNOS using 9D10 the antibody (1∶1 000) from Zymed Laboratories Inc., for hsp90 using H38220 (1∶1 000) and P-eNOS (S1177, human) using anti-P-eNOS (S1177). Autoradiograms were obtained using horseradish peroxidase (HRP) - linked secondary antibodies and chemiluminesence. Images of the bands of interest in the autoradiograms were obtained with UMAX Magicscan v4.4 and Adobe PhotoShop v5.5 software (UMAX Data Systems, Inc, Taipei, Taiwan).

1.2.5 Statistical Analysis All data are presented as the mean ± SD($\bar{x} \pm s$). Nitrite + nitrate data were examined by paired T test and superoxide anion data were examined by paired sample ANOVA. The Newman-Kuel's post-hoc test was employed to determine the level of significance between means. The minimum level of significance was set at $P < 0.05$.

2 Results

2.1 TRI Inhibits NO Generation

TRI inhibited A23187-stimulated nitrite + nitrate production by confluent BCEC from (1.16 ± 0.15) nmol · mg^{-1} to (0.91 ± 0.3) nmol · mg^{-1} (Table 1, $P < 0.05$).

2.2 TRI Increases O_2^- Generation by eNOS and Xanthine Oxidase

In contrast with the effects TRI on nitrite + nitrate production, TRI increased the release of O_2^- by A23187-stimulated confluent BCEC cultures from (0.015 ± 0.007) nmol/(min · mg) to (0.057 ± 0.022) nmol/(min · mg) (Table 2, $P < 0.05$). *L*-NAME, a substrate analogue inhibitor of NOS, increased the release of O_2^- from A23187-stimulated control cultures from (0.015 ± 0.007) nmol/(min · mg) to (0.044 ± 0.008) nmol/(min · mg) (Table 2, $P < 0.05$), confirming that under normal conditions eNOS in control cells generates NO that scavenges intracellular O_2^-(before it has a chance to escape from the endothelial cell and react with ferricytochrome c [14]. In A23187-stimulated cultures, exposed to TRI however, *L*-NAME decreased the release of O_2^- (0.057 ± 0.022) nmol/(min · mg) to (0.039 ± 0.005) nmol/(min · mg) (Table 2, $P < 0.05$) and oxypurinol, a competitive inhibitor of xanthine

Table 1 Effects of TRI on A23187-stimulated NO production

($\bar{x} \pm s$)

Groups	n	b(NO) / (nmol · mg^{-1})
Control	6	1.16 ± 0.15
TRI	6	0.91 ± 0.3 [1)]

1) Compared with control, $P < 0.05$

oxidase, decreased A23187-stimulated O_2^- production to (0.034 ± 0.012) nmol/(min · mg) (Table 2, $P < 0.05$), indicating that TRI induced confluent BCEC cultures to generate O_2^- by part of an eNOS-dependent mechanism and part of xanthine oxidase mechanism. Calculation of the relative change in O_2^- generation due to *L*-NAME and oxypurinol inhibition reveals the extent to which TRI induces confluent BCEC to generate O_2^- can be blocked by *L*-NAME plus oxypurinol. The increase of TRI on O_2^- generation: TRI-control = 0.057 − 0.015 = 0.042 nmol/(min · mg); The inhibition of generation due to *L*-NAME and oxypurinol on TRI-treated EC culture[nmol/(min · mg)]: [TRI-(O_2^-TRI + L − NAME)] + [TRI − (TRI + oxypurinol)] = (0.057 − 0.039) + (0.057 − 0.034) = 0.041nmol/(min · mg).

2.3 Effects of TRI on Expression of eNOS and hsp90 and on Phosphorylation of eNOS at S1179

Table 2 Effects of TRI on A23187-stimulated O_2^- generation

($\bar{x} \pm s$)

Groups	n	z/m(O_2^-) / nmol/(min · mg)
Control	10	0.015 ± 0.007
L-NAME	10	0.044 ± 0.008 [1)]
TRI	10	0.057 ± 0.022 [1)]
TRI + L-NAME	10	0.039 ± 0.005 [2)]
TRI + oxypurinol	10	0.034 ± 0.012 [2)]

1) Compared with control, $P < 0.05$. 2) Compared with TRI, $P < 0.05$

Immunoblots of control and TRI-treated cells revealed that TRI had no effect on the expression of eNOS (Fig. 1, middle panel: lane 2 *vs* lane 1, lane 4 *vs* lane 3) or hsp90 (Fig. 1, low panel: lane 2 *vs* lane 1, lane 4 *vs* lane 3) in confluent BCEC cultures or on the phosphorylation of eNOS (S1179) (Fig. 1, top panel: lane 2 *vs* lane 1, lane 4 *vs* lane 3) in

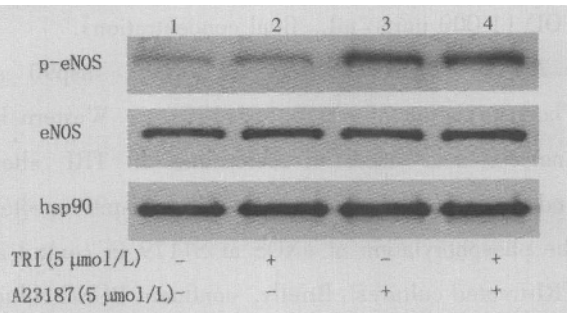

Fig. 1 Effects of TRI on p-eNOS, eNOS and hsp90 expression

unstimulated or A23187-stimulated cultures relative to controls (Fig. 1).

2.4 Effects of TRI on Association of Hsp90 with eNOS and Phosphorylation of eNOS at S1179

To determine the effects of TRI on eNOS protein interactions, eNOS was immunoprecipitated from unstimulated and A23187-stimulated control and TRI-treated BCEC cultures. Immunoblots for P-eNOS, eNOS and hsp90 on the immunoprecipitated eNOS complex revealed that A23187-stimulation increased P-eNOS (S1179) levels compared with the levels detected in unstimulated control cultures (Fig. 2, top panel: lane 3 *vs* lane 1). Pretreatment with TRI alone had little effect on the levels of P-eNOS (S1179) in unstimulated or A23187-stimulated BCEC cultures compared with their corresponding controls (Fig. 2, top panel: lane 2 *vs* lane 1, lane 4 *vs* lane 3). A23187 increased the association of hsp90 with eNOS compared with controls as described before[6] (Fig. 2, low panel: lane 3 *vs* lane 1) and TRI had

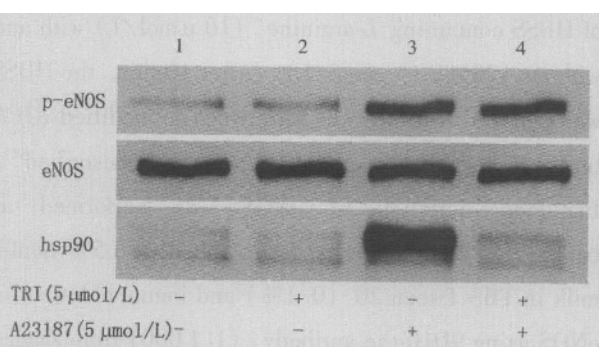

Fig. 2 Effects of TRI on the association of eNOS with hsp90

Top panel: phosphorylation of eNOS (S1179). Middle panel: the immunoprecipitated eNOS. Low panel: hsp90 associated with eNOS

little effect on the association of hsp90 with eNOS compared with controls in unstimulated cultures[6] (Fig. 2, low panel: lane 2 *vs* lane 1). However, TRI decreased the association of hsp90 with eNOS compared to control levels in A23187-stimulated BCEC cultures (Fig. 2, low panel: lane 4 *vs* lane 3) without increasing P-eNOS levels (Fig. 2, top panel: lane 4 *vs* lane 3).

3 Discussion

Our recent study showed that TRI shifted the balance of NO and O_2^- in proliferating EC by decreasing the association of hsp90 with eNOS without impairing the ability of EC to phosphorylate eNOS at S1179, and finally altered endothelial and eNOS function to impair VEGF-stimulated endothelial proliferation[10]. In this study, we also found that TRI altered the balance of NO and O_2^- generation on confluent BCEC, inhibited hsp90 association of eNOS with without significantly inhibiting the phosphorylation of eNOS at S1179, a change in protein interaction that allows eNOS to generate O_2^- [6, 15]. Findings in confluent BCEC are similar to our previous report and suggest that TRI disrupts hsp90 interactions with eNOS to induce endothelial and eNOS dysfunction. Such changes in radical species generation may play a role in endothelial dysfunction and cardiac teratogenicity.

As EC proliferation is characterized by marked increases in eNOS-dependent · NO production and NADPH oxidoreductase-dependent O_2^- generation[11, 12]. During proliferation EC enhance eNOS expression 2 to 3 fold. These observations suggest that eNOS activity in proliferating EC cultures must be highly coupled to L-arginine metabolism to maintain such increased level of NO production. Thus, eNOS activity in proliferating EC is different from eNOS activity in confluent EC. Here we also observed that in confluent BCEC, although as in proliferating BCEC TRI inhibits NO production and increases O_2^- generation, *L*-NAME only inhibited part of O_2^- generation from TRI – treated BCEC. However, oxypurinol inhibited a little more O_2^- generation than *L*-NAME from TRI-treated BCEC. Interestingly, the total inhibition of O_2^- generation by *L*-NAME and oxypurinol in TRI-treated confluent BCEC is essentially the same as TRI increased O_2^- generation by control EC, indicating that TRI not only uncouples eNOS activity to increase O_2^- generation but also it activates a second oxidative enzyme, xanthine oxidase, to increase O_2^- generation. Our previous report showed that native low-density lipoprotein (nLDL) increased O_2^- by an eNOS – dependent mechanism, whereas minimally oxidized LDL (mmLDL) increased O_2^- by eNOS –, xanthine oxidase- and NADPH oxidoreductase-dependent mechanisms in vascular endothelium of intact arterial segments[16]. Findings here are similar to these previous reports and suggest TRI increases O_2^- by an eNOS-dependent mechanism in proliferating EC, whereas by eNOS- and xanthine oxidase-dependent mechanisms in confluent EC. Our study suggests that in confluent EC, TRI induces endothelial and eNOS dysfunction by different mechanisms rather than those in proliferating EC.

Boyer reported TRI could regulate the expression of more than 40 cellular proteins[2]. Our previous findings revealed that TRI had no effects on the expression of eNOS or hsp90 but rather alters hsp90 interactions with eNOS in proliferating EC[10]. Here the above results also show that exposure to TRI altered hsp90 interactions with eNOS without altering expression of hsp90 or eNOS in confluent BCEC. Such changes in protein interactions underscore the importance of hsp90 in promoting coupled eNOS activity.

Compared with our previous report of TRI on proliferating EC, we found that although TRI decreased the association of hsp90 with eNOS more in confluent EC, it also increased eNOS-dependent O_2^- generation to a lesser extent in confluent BCEC. Content of eNOS is more 2 – 3 time in proliferating BCEC Cultures than in confluent BCEC Cultures[9]. Whether EC are confluent or proliferating does not appear to significantly effect hsp90 expression[9]. However, proliferating EC cultures contain 4 ~ 5 more hsp90 associated with eNOS than confluent EC cultures. Thus it is possible that TRI decrease hsp90 associated with eNOS more in confluent EC than in proliferating EC.

In addition, recently we found xanthine oxidase, one of major sources of O_2^-, can inhibit hsp90 interaction with eNOS to limit NO production[17] . Findings here show that TRI increase O_2^- generation by eNOS- and xanthine oxidase-dependent mechanisms in confluent BCEC, indicating that TRI induces endothelial and eNOS dysfunction by directly disrupting hsp90 interaction with eNOS and indirectly activating xanthine oxidase firstly, which further turn to alter hsp90 interaction with eNOS.

Over the last several years we have described how hsp90 regulated eNOS to influence vascular EC function and physiology[6, 7, 9, 10, 17-19] . In persistent pulmonary hypertension, disruption of hsp90 interaction with eNOS leads to impaired ATP-stimulated NO production[18] . Chronic hypoxia increases cardioprotection of isolated hearts against ischemia by increasing hsp90 association with eNOS which reduces eNOS-dependent superoxide by ~ 3 fold[19] . As the balance of NO and O_2^- is central to vascular biology, disturbances in this balance are believed to play a causal role in the genesis of ischemic heart disease due to atherosclerosis, diabetes and hypertension. Many cardiovascular diseases such as hypertension, coronary artery disease, atherosclerosis even cardioprotection during cardiac surgery are more influenced by changes in confluent EC function. Findings here indicate that exposure to TRI induces phenotypic changes in EC function that could promote cardiovascular disease.

In conclusion, TRI shifts the balance of NO and O_2^- in confluent BCEC by activating xanthine oxidase and by decreasing association of hsp90 with eNOS without impairing the ability of EC to phosphorylate eNOS at S1179 and without altering expression of hsp90 or eNOS. Such changes in eNOS and endothelial function may contribute to many cardiovascular diseases such as hypertension, coronary artery disease, atherosclerosis even impair cardioprotection during cardiac surgery.

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