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Effect of magnesium lithospermate B on calcium and nitric oxide in endothelial cells upon hypoxia/reoxygenation[1]

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KEY WORDS magnesium lithospermate B; endothelium; anoxia; calcium; nitric oxide

ABSTRACT

AIM: To investigate the effect of magnesium lithospermate B (MLB) on hypoxia/ reoxygenation (H/R)-induced elevation of intracellular calcium concentration ($[Ca^{2+}]_i$) and nitric oxide (NO) release in endothelial cells. **METHODS:** The cultured human umbilical vein endothelial cells (ECV304) were exposed to hypoxia for 30 min under 95 % N_2 and 5 % CO_2, then reoxygenation for 30 min under air and 5 % CO_2. Cell injury was evaluated by dye exclusion test, superoxide dismutase (SOD) assay, and molondialdehyde (MDA) assay. $[Ca^{2+}]_i$ was determined by Fura 2-AM. NO content was examined by a NO assay kit. Endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) mRNA expressions were measured by semi-quantitative RT-PCR. **RESULTS:** Cell viability was decreased from (93.1±1.2) % in normoxia to (88±3) % in H/R (P<0.01), and SOD activity was also decreased from (0.24±0.07) kNU/L to (0.18±0.03) kNU/L in H/R (P>0.05), but MDA production was increased from (1.12±0.06) mmol/L in normoxia to (3.78±0.03) mmol/L in H/R (P<0.01) in ECV304 cultured under calcium conditions. MLB 2.5, 5, and 10 mg/L increased cell viability and SOD activity, and inhibited MDA formation in ECV304. H/R increased $[Ca^{2+}]_i$ (F_{340}/F_{380} from 1.65±0.16 to 1.89±0.28), NO release [from (7.5±1.3) μmol/L to (16±5) μmol/L], and eNOS mRNA expression, but decreased iNOS mRNA expression in ECV304 (P<0.05). However, it did not affect them under calcium-free conditions. MLB inhibited H/R-induced increases in $[Ca^{2+}]_i$ and eNOS mRNA expression, stimulated NO release and iNOS mRNA expression (P<0.05). **CONCLUSION:** MLB attenuates H/R-induced cell injury and increases NO release in ECV304. This increase of NO production is possibly associated with preventing cell injury induced by H/R in MLB-treated ECV304.

INTRODUCTION

Endothelial cells (EC), which are the essential constituent of vessel, are known to modulate vascular reactivity by releasing vasoactive substances, such as nitric oxide[1]. Nitric oxide (NO) is an important potent mediator in the cardiovascular system, as well as in nervous and immune system[2-4]. NO is synthesized in the endothelium by two major nitric oxide synthases (NOS), namely eNOS, which is calcium/calmodulin dependent, and iNOS, which is not calcium/calmodulin dependent[5,6]. NO is a principal mediator in various pathological processes, and several investigates have indicated that NO plays a key role in hypoxia/ reoxygenation (H/R). During H/R, EC was damaged,

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and NO synthesis was given rise to malfunction[7,8].

Calcium is also an important second mediator in various cells. Intracellular calcium concentration ($[Ca^{2+}]$) in EC plays an important role in regulating a variety of cellular functions, such as affecting eNOS mRNA expression[3,9,10]. The change of $[Ca^{2+}]_i$ is mediated by voltage- or ligand-gated Ca^{2+} channels that regulate Ca^{2+} influx across the plasma membrane and/or by ligand-gated Ca^{2+} channels which control the release of Ca^{2+} from intracellular stores[11]. Hypoxia stimulated extracellular calcium influx, but $[Ca^{2+}]_i$ was decreased during reoxygenation in EC[12].

Magnesium lithospermate B (MLB), a biologically active compound, is newly extracted from a well-known Chinese herb Radix Salviae Miltiorrhizae. Several investigators have found that MLB could improve renal function via the killikrein-prostaglandin system in rats with renal failure[13]. Kamata *et al* found that MLB could endothelium-dependently relax the noradrenaline (NE)-precontracted aorta[14]. Our previous studies indicated that MLB could attenuate myocardial ischemia-reperfusion injury in SD rats *in vivo*, and prevented hypoxia-induced extracellular calcium influx and NO release in endothelial cells[15]. But until now we have unknown about the effect of MLB in the aspects of H/R. In the present study, we aimed to investigate the effect of MLB on H/R-induced cell injury, $[Ca^{2+}]_i$, NO release, and eNOS and iNOS mRNA expressions in endothelial cells, in order to explore the mechanisms of the protective effect of MLB on ischemia/reperfusion injury.

MATERIALS AND METHODS

Cell culture ECV304, a human umbilical vein endothelial cell line, was obtained from Cell Bank of Chinese Academic of Science. The culture medium was Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10 % bovine calf serum, benzylpenicillin 100 kU/L, streptomycin sulfate 100 kU/L, HEPES 4.7 mmol/L, and sodium bicarbonate 44 mmol/L. The cells were incubated in a 37 ℃ humidified incubator gassed with 5 % CO_2.

Drugs and reagents MLB, a gift from Prof XU Ya-Ming (Department of Phytochemistry, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences), was a yellow powder, purity >95 %. It was dissolved in dH_2O. Krebs' solution containing calcium (mmol/L): NaCl 120, KCl 5.6, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, glucose 10, and $CaCl_2$ 2.5; calcium-free Krebs' solution (mmol/L): NaCl 120, KCl 5.6, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, glucose 10, and egtazic acid 0.5. DMEM (low glucose), TRIzol and M-MLV reverse transcriptase were purchased from Gibco (NY, USA). Fura 2-AM and BSA were purchased from Sigma (MO, USA). *Taq* polymerase was purchased from Sangon (Shanghai, China). NO, SOD, and MDA assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other reagents were of AR grade.

Hypoxia/reoxygenation protocol The resulting subconfluent monolayers of ECV304 were digested with 0.125 % trypsin (w/v) and 0.04 % edetic acid (w/v) in *D*-Hanks' solution, and seeded at 5×10^8/L onto 24-well dishes or at 1×10^9/L onto 6-well dishes. After overnight, the ECV304 were rinsed twice with *D*-Hanks' solution, and incubated in 1 mL Krebs' solution containing calcium and calcium-free Krebs' solution with 1 % bovine serum albumin (BSA, w/v), respectively. Then the dishes were placed at a sealed chamber, and bubbled with 95 % N_2 and 5 % CO_2 for 30 min hypoxia, then reoxygenation 30 min under air and 5 % CO_2 at 37 ℃.

Cell viability After H/R, cell injury was assessed by erythrosine B uptake after incubation with 0.4 % erythrosine B in PBS for 5 min, as previously described[15]. At least 300 cells were counted for each determination. Cells unable to exclude the colorant were considered to be nonviable. Erythrosine B staining only occurred in irreversibly injured cells.

Assay of MDA and SOD After H/R, the medium was collected, and the amount of molondialdehyde (MDA) and superoxide dismutase (SOD) released by cells were determined using MDA and SOD assay kits according to the manufacturer's protocol, respectively. The absorbance of samples was read at 532 and 550 nm, respectively.

Measurement of $[Ca^{2+}]_i$ $[Ca^{2+}]_i$ was measured with Fura 2-acetoxymethyl ester (Fura 2-AM), as previously described[15]. Briefly, the confluent monolayers of ECV304 in 24-well dishes were preloaded with Fura 2-AM. The final concentration of Fura 2-AM was 2 μmol/L. Then cells were incubated under H/R as described above. After H/R, the cell suspension was transferred to a quartz cuvette at 37 ℃ on a fluorescence spectrophotometer (HITACHI 650-10 S, Japan), and fluorescence was measured with emission wavelength at 510 nm and excitation wavelength respectively at 340

nm and 380 nm. The fluorescence ratio (F_{340}/F_{380}) was calculated as an indicator of $[Ca^{2+}]_i$.

Assay of nitrite and nitrate After H/R, the medium was collected, and the amount of NO released by cells was determined using a NO assay kit according to the manufacturer's protocol. The method involved measuring the amount of NO metabolites (nitrite and nitrate), which were more stable than NO. The absorbance of samples was read at 550 nm.

Reverse transcription-polymerase chain reaction (RT-PCR)[15] After H/R, total RNA from cultured cells was extracted with TRIzol (Life Technologies, Gibco). The PCR primers were as follows: eNOS, sense 5'-GTG ATG GCG AAG CGA GTG AAG-3'; antisense 5'-CCG AGC CCG AAC ACA CAG AAC-3'; iNOS, sense 5'-CGG TGC TGT ATT TCC TTA CGA GGC GAA GAA GG-3'; antisense 5'-GGT GCT GCT TGT TAG GAG GTC AAG TAA AGG GC-3'; and GAPDH, sense 5'-CCA TGG AGA AGG CTG GGG-3'; antisense 5'-CAA AGT TGT CAT GGA TGA CC-3'. The cDNA was synthesized using oligo-dT_{18} (0.5 g/L) from 1 μg of total RNA. cDNA 2 μL was used for PCR with magnesium chloride 1.5 mmol/L, dNTPs 10 mmol/L, primer 25 μmol/L, 10×PCR buffer [Tris-HCl (pH 9.0) 100 mmol/L, KCl 100 mmol/L, $(NH_4)_2SO_4$ 80 mmol/L, NP-40] and 2.5 unit *Taq* polymerase in a final volume of 50 μL. Touch-down PCR cycles for eNOS were performed as following: denaturation at 95 ℃ for 30 s and annealing temperature decreasing 1 ℃ every second cycle from 70 ℃ to a touch-down at 60 ℃, then denaturation at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s and extension at 72 ℃ for 30 s for 30 cycles. PCR cycles for iNOS were performed as following: denaturation at 95 ℃ for 5 min and 94 ℃ for 45 s, annealing at 60 ℃ for 45 s and extension at 72 ℃ for 2 min for 32 cycles, and an additional 7 min extension at 72 ℃. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was carried out on the same samples as a parallel control. GAPDH PCR was performed as following: denaturation at 95 ℃ for 5 min and 94 ℃ for 30 s, annealing at 55 ℃ for 30 s and extension at 72 ℃ for 30 s for 25 cycles. The PCR products were separated by electrophoresis on a 2 % agarose gel containing ethidium bromide and visualized under UV light. Identity was confirmed by a gel documentation system (FR-980), and the values were expressed as ratios of eNOS to GAPDH and iNOS to GAPDH.

Statistical analysis All data were expressed as mean±SD, and analyzed by one-way ANOVA. $P<0.05$ was considered significant.

RESULTS

Effects of MLB on hypoxia/reoxygenation-induced mortality in endothelial cells The influence of MLB on cell viability in normal conditions (21 % O_2) was first analyzed in order to determine the *in vitro* toxicity of this molecule on EC. The cultured EC on 6-well dishes incubated under normoxia in the presence of different concentrations of MLB from 2.5 to 10.0 mg/L. Cell viability was evaluated by a dye exclusion test. The results showed that MLB did not inhibit EC growth, even at high concentration (10 mg/L), MLB did not interfere with the growth of EC incubated in normal conditions.

In order to evaluate H/R-induced cell injury in EC, cell viability was determined by a dye exclusion test and expressed as percentage of erythrosine B uptake after 30 min hypoxia and 30 min reoxygenation. In Krebs' solution containing calcium, cell viability rate was reduced to 88 % after H/R. Compared with normoxia control (93.1 %), the difference was significant ($P<0.05$). In the presence of MLB at concentrations of 2.5, 5, and 10 mg/L, MLB significantly attenuated cell injury induced by H/R, cell viability rates were increased by 90 % ($P>0.05$), 91.6 %, ($P<0.05$), and 91.2 % ($P>0.05$), respectively. In calcium-free Krebs' solution, cell injury greatly increased compared with Krebs' solution containing calcium ($P<0.01$). But the values of each group were similar ($P>0.05$, Fig 1).

Effects of MLB on hypoxia/reoxygenation-induced MDA formation in endothelial cells In Krebs' solution containing calcium, H/R obviously induced MDA formation in EC. Its value reached (3.78±0.03) mmol/L from (1.12±0.06) mmol/L in normoxia control ($P<0.01$). MLB 10 mg/L obviously inhibited MDA formation, and its MDA value was (3.45±0.09) mmol/L ($P<0.01$). But MLB 2.5 and 5 mg/L had no effects on it, and the values were (3.82±0.07) mmol/L and (3.77±0.09) mmol/L, respectively ($P>0.05$). In calcium-free conditions, H/R had no effects on MDA formation, and the MDA values were similar ($P>0.05$, Fig 2).

Effect of MLB on hypoxia/reoxygenation-induced SOD production in endothelial cells In Krebs' solution containing calcium, H/R reduced SOD

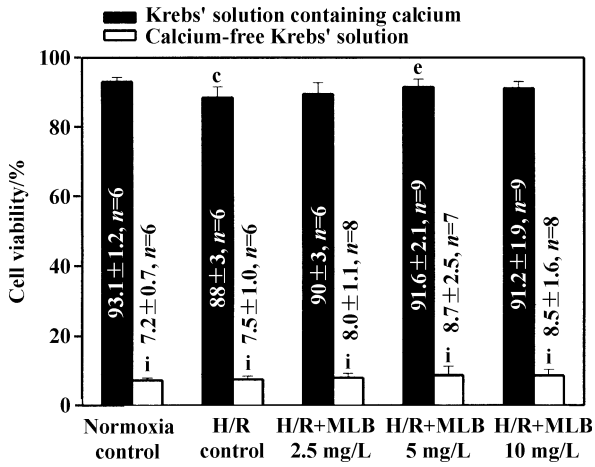

Fig 1. Effect of MLB on H/R-induced cell viability in ECV304. Mean±SD. [c]*P*<0.01 *vs* normoxia control. [e]*P*<0.05 *vs* H/R control. [i]*P*<0.01 *vs* Krebs' solution containing calcium.

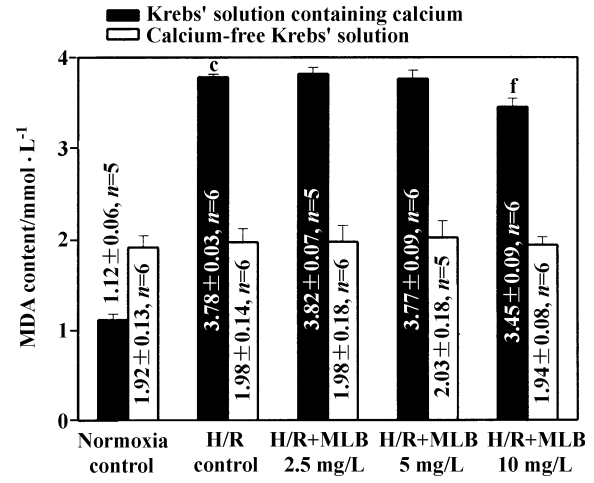

Fig 2. Effect of MLB on H/R-induced MDA generation in ECV304. Mean±SD. [c]*P*<0.01 *vs* normoxia control. [f]*P*<0.01 *vs* H/R control.

activity from (0.24±0.07) kNU/L in normoxia control to (0.18±0.03) kNU/L (*P*>0.05). MLB at final concentrations of 2.5, 5, and 10 mg/L obviously enhanced SOD activity in EC, and the SOD values were (0.29±0.07), (0.39±0.11), and (0.43±0.06) kNU/L (*P*<0.01). In calcium-free conditions, there was no SOD activity in EC (Tab 1).

Tab 1. Effect of MLB on H/R-induced SOD activity in ECV304. *n*=6. Mean±SD. [c]*P*<0.01 *vs* H/R control.

Group	Calcium condition (kNU/L)	Calcium-free condition (kNU/L)
Normoxia control	0.24±0.07	0.00±0.00
H/R control	0.18±0.03	0.00±0.00
H/R+MLB (2.5 mg/L)	0.29±0.07[c]	0.00±0.00
H/R+MLB (5 mg/L)	0.39±0.11[c]	0.00±0.00
H/R+MLB (10 mg/L)	0.43±0.06[c]	0.04±0.09

Effects of MLB on hypoxia/reoxygenation-induced $[Ca^{2+}]_i$ increase in endothelial cells In Krebs' solution containing calcium, the fluorescence ratio (F_{340}/F_{380}) for normoxic cells was 1.65±0.16, while it reached 1.89±0.28 in H/R control (*P*<0.01). MLB at final concentrations of 2.5, 5, and 10 mg/L prevented H/R-induced an increase of $[Ca^{2+}]_i$, the F_{340}/F_{380} were

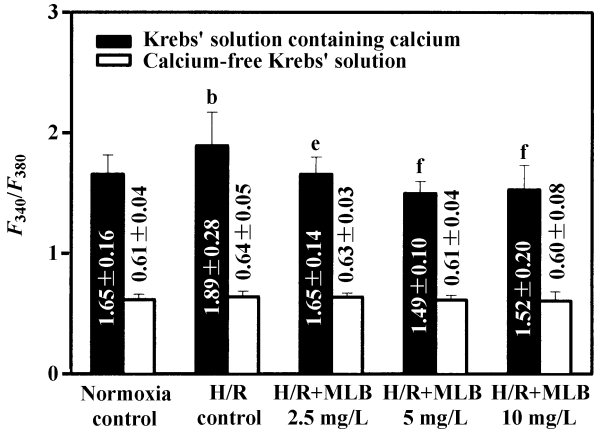

Fig 3. Effect of MLB on H/R-induced $[Ca^{2+}]_i$ in ECV304. *n*=6. Mean±SD. [b]*P*<0.05 *vs* normoxia control. [e]*P*<0.05, [f]*P*<0.01 *vs* H/R control.

1.65±0.14 (*P*<0.05), 1.49±0.10 (*P*<0.01) and 1.52±0.20 (*P*<0.01), respectively. In calcium-free Krebs' solution, the fluorescence ratio decreased in compare to Krebs' solution containing calcium (*P*<0.01). But H/R could not elicit an increase of $[Ca^{2+}]_i$ in calcium-free Krebs' solution, and MLB at different concentrations of 2.5 to 10 mg/L could not decrease $[Ca^{2+}]_i$ either. The F_{340}/F_{380} of each group were 0.61±0.04, 0.64±0.05, 0.63±0.03, 0.61±0.04, and 0.60±0.08, respectively (*P*>0.05, Fig 3).

Effects of MLB on hypoxia/reoxygenation-induced NO release in endothelial cells In Krebs' solution containing calcium, H/R significantly stimulated NO release in EC. The amount of NO released into the culture medium was (15±4) μmol/L in H/R control (*P*<0.01), while the NO content was (7.5±1.3) μmol/L in normoxia control. MLB at concentrations of 2.5, 5, and 10 mg/L enhanced NO release, and the NO contents were (16±5) (*P*>0.05), (20±10) (*P*<0.05), and

(21±4) μmol/L (P<0.01), respectively. In calcium-free Krebs' solution, H/R had no effects on NO release in EC, nor did MLB at different concentrations of 2.5 to 10 mg/L. The NO contents of each group were (10±4), (10±3), (10±3), (10±6), and (10±4) μmol/L, respectively. They had no statistically significance (P>0.05, Fig 4).

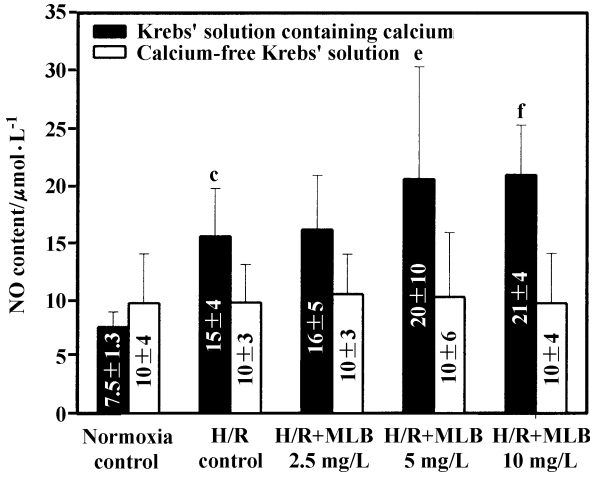

Fig 4. Effect of MLB on H/R-induced NO production in ECV304. n=6. Mean±SD. ^{c}P<0.01 *vs* normoxia control. ^{e}P<0.05 , ^{f}P<0.01 *vs* H/R control.

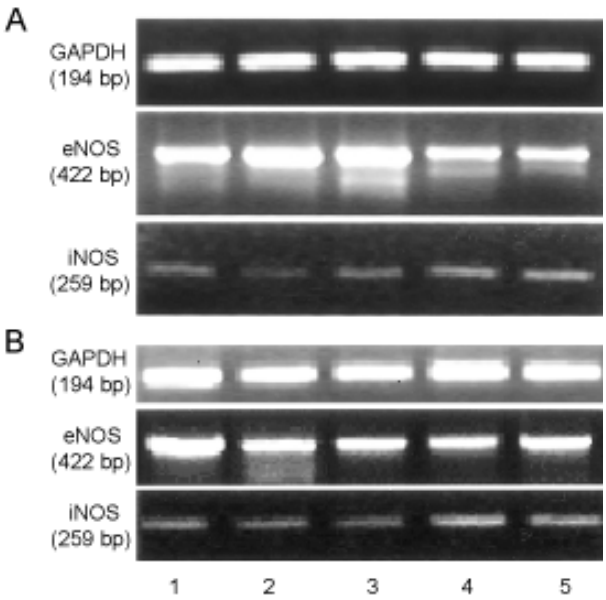

Fig 5. Effect of MLB on H/R-induced eNOS and iNOS mRNA expression in ECV304. The expression of eNOS, iNOS and GAPDH mRNA was determined by RT-PCR analysis. PCR products were run and separated by 2 % agarose gel. (A): the ECV304 cultured in Krebs' solution containing calcium; (B): the ECV304 cultured in calcium-free Krebs' solution. Lane 1: normoxia control; lane 2: H/R control; lane 3: H/R+MLB 2.5 mg/L; lane 4: H/R+MLB 5 mg/L; lane 5: H/R+MLB 10 mg/L.

Effects of MLB on hypoxia/reoxygenation-induced eNOS and iNOS mRNA expression in endothelial cells As shown in Fig 5, in Krebs' solution containing calcium, H/R up-regulated eNOS mRNA expression, but down-regulated iNOS mRNA expression compared with normoxia control. MLB down-regulated the eNOS mRNA expression, and up-regulated iNOS mRNA expression in a concentration-dependent manner. In the presence of MLB at concentrations of 2.5, 5, and 10 mg/L, the ratios of eNOS to GAPDH mRNA were 0.8±0.4, 0.55±0.13, and 0.60±0.08, respectively; but the ratios of iNOS to GAPDH mRNA were 0.43±0.27, 1.12±0.28, and 1.24±0.27, respectively. In calcium-free Krebs' solution, the ratios of eNOS and iNOS mRNA to GAPDH mRNA of each group was similar and not significant (P>0.05, Tab 2).

DISCUSSION

In the present study, we found that H/R increased MDA formation, and induced cell death in endothelial cells. It has been shown to be rapidly harmful for endothelial cell, to generate a burst of ROS, in particular superoxide anion (O_2^-), and to induce cellular calcium overload during I/R[7]. During H/R, O_2^- reacted with equimolar amount of NO, formed peroxynitrite ($ONOO^-$) in the irreversible manner, then cell released many hydroxyl free radicals ($^{\cdot}HO$), which have cell toxic effect. So the MDA generation was increased and SOD activity was decreased in EC upon H/R. After MLB treatment, MLB enhanced SOD activity, removed O_2^- effectively, and alleviated cell lipid peroxide injury.

Overloading calcium will elicit a series of signal transduction processes, induce mitochondrion to produce free radicals[7], and trigger cell death[16]. Our previous study indicated that MLB could significantly prevent $[Ca^{2+}]_i$ from increasing in EC upon hypoxia. In this study, we also found the same results, and MLB significantly inhibited extracellular calcium influx, and attenuated cell injury. Also, the expression of eNOS mRNA which is the strictly calcium/calmodulin dependent enzyme also down-regulated due to the decrease of $[Ca^{2+}]_i$.

However, NO is also a beneficial agent in I/R. It

Tab 2. Effect of MLB on H/R-induced eNOS and iNOS mRNA expression in ECV304. The ratios of A_{eNOS}/A_{GAPDH} and A_{iNOS}/A_{GAPDH} were used to express the levels of eNOS and iNOS mRNA in ECV304, respectively. *n*=2. Mean±SD. $^{c}P<0.01$ *vs* normoxia control. $^{e}P<0.05$ *vs* H/R control.

Group	eNOS		iNOS	
	Calcium	Calcium-free	Calcium	Calcium-free
Normoxia control	0.92±0.02	0.94±0.06	0.815±0.021	0.92±0.04
H/R control	1.16±0.09	0.83±0.25	0.21±0.05^{c}	0.94±0.08
MLB 2.5 mg/L	0.8±0.4	0.74±0.10	0.43±0.27	1.1±0.4
MLB 5 mg/L	0.55±0.13^{e}	0.68±0.17	1.12±0.28^{e}	1.19±0.11
MLB 10 mg/L	0.60±0.08^{e}	0.73±0.27	1.24±0.27^{e}	0.98±0.07

enhances vascular relaxation, increases diastolic distensibility in a range of different hearts, and inhibits expressions of some adhesion molecules[7]. In the present study, MLB significantly enhanced NO production, which attenuated cell injury. NO has been reported to reduce calcium overload in ischemia/reperfusion injury[17], which suggested that MLB inhibited extracellular calcium influx via enhancing NO release in endothelial cells upon hypoxia/reoxygenation. In addition, we found that the increase in NO production was mainly caused by iNOS mRNA overexpression. Although iNOS is expressed under the inflammatory stimulus, it also serves as a vasoprotective agent by inhibiting platelet aggregation, leukocyte chemotaxis, VSMC proliferation, VSMC migration, and by promoting endothelial cell survival and growth[18]. In our previous studies, MLB could inhibit hypoxia-induced the increase of iNOS mRNA expression in EC. But in present study, we found that MLB could stimulate its expression in the dose-dependent manner in EC upon H/R. These results suggest that iNOS plays the different function between hypoxia and H/R, and during H/R, iNOS overexpression exerts beneficial effect. Also, iNOS attenuated peroxynitrite generation[19]. Kanno *et al* has reported that superinduction of iNOS attenuated myocardial ischemia/reperfusion[20].

In conclusion, our results indicated that MLB could inhibit extracellular calcium influx, down-regulate eNOS mRNA expression, up-regulate iNOS mRNA expression, and stimulate NO production. The effect of MLB on attenuating cell injury induced by H/R is associated with the increase of NO production in EC. It suggests that MLB have protective effect on ischemia/reperfusion at cellular levels.

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丹酚酸B镁盐对缺氧复氧内皮细胞内钙和一氧化氮的影响[1]

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关键词　丹酚酸B镁盐；内皮；低氧；钙；一氧化氮

目的：研究丹酚酸B镁盐对缺氧复氧引起的内皮细胞内钙升高和一氧化氮释放增加的影响. **方法**：培养的人脐静脉内皮细胞(ECV304)暴露在95 % N_2+5 % CO_2条件下缺氧30分钟，后在含5 % CO_2的空气中复氧30分钟. 内皮细胞的损伤用染料排除实验、SOD的活性和MDA的生成来评价. 胞内游离钙浓度用钙荧光探针Fura 2-AM 测定. 一氧化氮含量用一氧化氮试剂盒测定. 内皮型一氧化氮合酶 (eNOS) mRNA和诱导型一氧化氮合酶(iNOS) mRNA的表达用半定量逆转录聚合酶链式反应(RT-PCR)检测. **结果**：缺氧复氧引起内皮细胞的活力由正常条件下(93.1 ± 1.2) %降至(88 ± 3) % (P<0.01)，SOD的活性也由(0.24 ± 0.07) kNU/L下降到(0.18 ± 0.03) kNU/L (P>0.05)，但其使内皮细胞MDA的生成由(1.12 ± 0.06) mmol/L增至(3.78 ± 0.03) mmol/L (P<0.01). 丹酚酸B镁盐2.5,5,10 mg/L能明显降低缺氧复氧引起的内皮细胞MDA生成量的增加，并且显著提高细胞活力和SOD的活性. 同时，缺氧复氧还增加内皮细胞的胞内游离钙浓度(F_{340}/F_{380}由1.65 ± 0.16增至1.89 ± 0.28)和一氧化氮释放[由(7.5 ± 1.3) μmol/L增至(16 ± 5) μmol/L]，并上调其eNOS mRNA的表达，但降低iNOS mRNA的表达(P<0.05). 但在无钙的条件下，缺氧复氧对内皮细胞的胞内游离钙浓度、一氧化氮含量、eNOS mRNA和iNOS mRNA表达均没有影响. 丹酚酸B镁盐能显著降低缺氧复氧引起的内皮细胞内钙升高和eNOS mRNA的表达，增加一氧化氮释放和iNOS mRNA的表达(P<0.05). **结论**：丹酚酸B镁盐能改善缺氧复氧引起的内皮细胞损伤，增加内皮细胞一氧化氮的释放. 丹酚酸B镁盐提高一氧化氮合成的这种机制可能与其改善内皮细胞缺氧复氧损伤有关.

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