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렙틴 수용체가 형질 도입된 신경세포에서 ER Stress로 유도된 렙틴저항성에 대한 일산화탄소의 억제작용

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Carbon Monoxide Prevents Ob-Rb Transfected Neuronal Cells from ER Stress-Induced Leption Resistance

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Abstract

Background: It is well known that leptin is an important circulating signal for the inhibition of food intake and the gain of body weight. Accumulating data suggest that leptin resistance has been considered to be one of the main causes of obesity. However, the detailed mechanism of leptin resistance is not known yet. Recently, endoplasmic reticulum (ER) stress-induced unfolded protein response (UPR) is reported to be responsible for the leptin resistance.
Materials & methods: Carbon monoxide (CO), a reaction product of the cytoprotective heme oxygenase (HO)-1, is anti-apoptotic in a variety of models of cellular injury. Exogenous CO activated Nrf2 through the phosphorylation of protein kinase R-like endoplasmic reticulum kinase (PERK), resulting in HO-1 expression. CO-induced activation of PERK was followed by the phosphorylation of eukaryotic translation initiation factor 2a and the expression of activating transcription factor 4. However, CO fails to induce X-box binding protein-1 expression and activating transcription factor 6 cleavage. CO prevented X-box binding protein-1 expression and activating transcription factor 6 cleavage induced by ER-stress inducer such as thasigargin (TG), tunicamycin (TM) and homocysteine.
Results: In the present study, we hypothesized that exogenous CO might block the leptin resistance which is induced by UPR during ER stress. Thasigargin or tunicamycin was used to induce ER stress. The activation status of leptin signals were measured by western blotting analysis using a phospho-signal transducer and activation of transcription3 (STAT3) antibody. In this study, ER stress markedly inhibited leptin-induced STAT3 phosphorylation.
Conculusion: These results suggest that ER stress induces leptin resistance and exogenous CO-induced phosphorylation of PERK branch reversed ER stress-induced leptin resistance. Moreover, CO releasing molecule (CORM) blocks the inhibition of leptin-induced STAT3 phosphorylation. Together, the pathological mechanism of leptin resistance could be ameliorated by the use of exogenous CO..

Key words : Endoplasmic reticulum (ER), Unfolded protein response(UPR), Carbonmonoxide(CO), Leptin resistance, phospho-signal transducer and activation of transcription3 (STAT3)

Ⅰ. INTRODUCTION

Leptin, a circulating hormone, is best known as a regulator of food intake and body weight gain through its actions in the brain.[1)] Leptin receptor (Ob-Rb) is the product of the diabetes (db) gene.[2)] However, binding of leptin to its receptor activates janus kinase 2 (JAK2). JAK2 activation leads to tyrosine phosphorylation of Ob-Rb. Signal transducers and activators of transcription 3 (STAT3) proteins bind to phosphorylated Tyr1138 of Ob-Rb, become tyrosine phosphorylated by JAK2, then dissociate and form dimers in the cytoplasm and finally translocate to the nucleus to regulate gene transcription. Because specific knockout (KO) of the Tyr1138 residue of

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Ob-Rb causes severe obesity in mice, the role of STAT3 activation is very important in the regulation of body weight by leptin.[3-5)]

Obesity is a condition with excess body fat which in turn may have an adverse effect on health. Leptin therapy could be an important tool of obesity treatment. Presently, leptin resistance is taking in account as one of the vital causes of obesity.[6)] Leptin resistance involves suppressors of cytokine signaling 3 (SOCS3).[7,8)] or protein tyrosine phosphatase 1B (PTP1B).[9)] The proteins have been shown to play important roles in the blockade of leptin signaling. Moreover, obesity-induced insulin resistance and type 2 diabetes can be mediated by endoplasmic reticulum (ER) stress and activation of unfolded protein response (UPR) signaling pathways.[10)] Many researchers have shown that ER stress plays a central role in development of leptin resistance.[11)] It is also noticed that the details of leptin resistance is not completely known.

The ER is a central organelle of each eukaryotic cell as the place of protein synthesis, maturation and protein folding. Perturbation of the above mentioned processes causes ER stress leading to activation of UPR.[12)] In mammalian cells, UPR is a complex signaling network, including 3 ER stress sensors: the double-stranded RNA-activated protein kinase (PERK), the inositol requiring enzyme 1 (IRE1), and the basic leucine-zipper activating transcription factor 6 (ATF6).[13)] Among the members of eukaryotic translation initiation factor (eIF) 2a kinase family, PERK remains at ER membrane responsible for ER stress signals. IRE1 transcription factor allows X-box binding protein (Xbp-1) to activate upstream ER-stress responding genes. In addition, expression of ER-resident chaperones such as the 78-kDa glucose-regulated protein (GRP) 78 and GRP94 responsible for ER protein folding is involved in increased levels of IRE1 and ATF6.[14)]

Carbon monoxide (CO) is a reaction product of heme oxygenase-1(HO-1) activity, as HO-1 helps the degradation reaction of the heme into CO, biliverdin, and free iron.[15,16)] Though CO was considered as a toxic waste product of heme catabolism, presently it is becoming as a crucial molecule for biological research. Further, CO has anti-inflammatory, anti-proliferative as well as anti-apoptotic effects on cells.[17-19)] A very recent report suggested that CO can modulate STAT3 activation via phosphatidylinositol 3-kinase/Akt and p38 MAPK pathways. CO-induced inhibition of Fas expression and caspase 3 activity is responsible for the anti-apoptotic effect in endothelial cells.[20)] Recently, Kim et al showed that ER stress-induced endothelial cell apoptosis could be blocked by CO via the inhibition of the expression of C/EBP homologous protein (CHOP).[21)] Thus, this study was designed to know whether CO also blocks the ER stress-induced leptin resistance.

Ⅱ. MATERIALS AND METHODS

1. Reagents

Tricarbonyl dichlororuthenium (II) dimmer (RuCO) were purchased from Sigma-Aldrich (St Louis, Mo, USA). Leptin was purchased from R&D systems (Minneapolis, MN, USA). Thapsigargin (TG), tunicamycin (TM) were obtained from Calbiochem (La Jolla, Calif, USA). Lipofectamine 2000 was purchased from Invitrogen Life Technology (Grand Island, NY, USA). Antibodies to phospho (p)-PERK, PERK, eIF2α, CHOP, OB-R,STAT-3 and ß-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, Calif, USA); and antibodies to p-STAT3 (Tyr705), p-eIF2α, were from Cell Signaling Technology (Beverly, Mass, USA). Antibodies to IRE1α were obtained Novus Biologicals (Littleton, CO, USA); and antibodies to GRP78 was purchased from Stressgen (Ann Arobor, MI, USA). The small interfering (si)RNAs against IRE1 were obtained from Santa Cruz Biotechnology. All other chemicals were obtained from Sigma-Aldrich.

2. Cell Culture

Chinese hamster CHO-K1 cells were maintained in Ham's F-12 Nutrient Mixture supplemented with 10% heat-inactivated fetal bovine serum maintained at 37° C in humidified 5% CO_2 and 95% air. Human neuroblastoma SK-N-AS cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum at 37° C in humidified 5% CO_2 and

95% air.

3. Generation of Ob-Rb Leptin Receptor-Stable Transfectant Cell Line

The Ob-Rb leptin receptor plasmid DNA was a kind gift from Dr. Sung-Kyu Ju. The Ob-Rb leptin receptor construct was transfected into SK-N-AS-Ob-Rb and CHO-K1 cells using lipofectamin reagent according to the manufacturer's instructions. After the transfection, stable transfectants were obtained by selection with the hygromycin.[26)]

4. Western Blot and Densitometry Analyses

After treatment, cells were harvested and washed twice with ice-cold PBS. Cells were lysed with 150 mM NaCl, 1.0% IGEPAL® CA-630, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, protease inhibitor cocktails, phophatase inhibitor cocktails for 20 minutes. The lysates were centrifuged at 13,000 rpm for 15 min at 4° C, and supernatants were collected. Protein content was measured with BCA protein assay reagent (Pierce). The samples were boiled with Laemmli buffer for 5 min, and equal amounts of protein (50 μg of protein) were separated on 7.5% to 12% SDS-PAGE and transferred to poly (vinylidene difluoride) membranes. The membranes were blocked with 5% nonfat milk in PBS containing 0.1% Tween 20 (PBS-T) for 30 minutes and incubated with antibodies to p-STAT3 (1:1000), p-PERK (1:500), p-eIF2α (1:1000), IRE1 (1:500), Ob-R (1:500), GRP78 (1:1000), CHOP (1:500), in PBS-T containing 1% nonfat milk for 3 hours. After washing 3 times with PBS-T, the membranes were hybridized with horseradish peroxidase-conjugated secondary antibodies for 40 minutes. Following 5 washes with PBS-T, they were incubated with chemiluminescent solution for 5 minutes and protein bands were visualized on x-ray film. For the densitometry analysis, optical density (the gray-scale value of pixels: 0 to 255) was measured on the inverted digital images using Scion Image (Scion Corp, Frederick, Md).

5. Transfection of siRNAs

Predesigned siRNAs against human IRE1 were purchased from Santa Cruz Biotechnology. Cells were transfected with double-stranded siRNAs (50nM) for 12 hours by the Lipofectamine method according to the protocol of the manufacturer (Invitrogen Life) and recovered in fresh media containing 10% FBS for 24 hours. The interference of IRE1 expressions was confirmed by immunoblot using anti-IRE1 antibodies; scrambled siRNA was used as a control.

6. Statistical Analyses

Data were expressed as mean ± SD. t-tests were used to assess significant differences between groups. A p value of >.05 was considered to represent a statistically nonsignificant change.

Ⅲ. RESULTS

1. CO Induces Activation of PERK Branch of UPR in ER Stress Response

It is reported that leptin resistance is induced by ER stress.[22)] So we induced ER stress by agents that interfere with protein glycosylation (i.e., tunicamycin; TM), Ca^{2+} balance (i.e., thapsigargin; TG). The conditions used for induction of ER stress trigger the cellular unfolded protein response (UPR), which results in an increase in UPR-regulated genes such as glucose-regulated protein 78 (GRP78), an ER-resident chaperone, or CHOP, an ER stress-induced apoptotic transcription factor. We examined effects of RuCO on ER stress response including PERK, GRP78 and CHOP. Firstly, we confirmed that TM and TG induced GRP78 and CHOP in CHO-K1 and SK-N-AS cell stably transfected with the Ob-Rb leptin receptor (CHO-K1-Ob-Rb and SK-N-AS-Ob-Rb, respectively). Also these ER stress inducer activate PERK pathway of ER stress response, and induce phosphorylation of PERK and eIF2 (Fig.1, B and C). On the other hand, RuCO, which is CO releasing molecules, activates PERK pathway, without affecting on expression of GRP78 and CHOP (Fig. 1A). These results suggest that CO is involved in ER stress response through activation of PERK pathway.

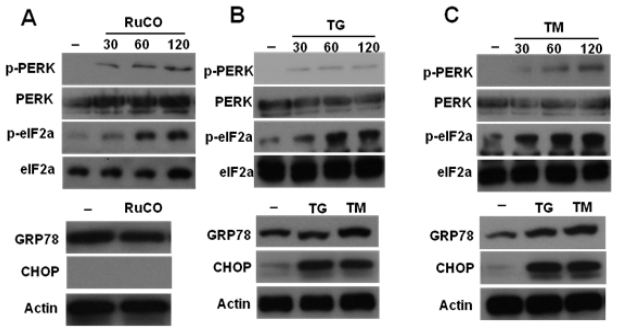

Fig. 1. CO induces activation of PERK branch of ER stress in CHO-K1-Ob-Rb and SK-N-AS-Ob-Rb cells.
(A) SK-N-AS-Ob-RB cells were incubated with RuCO (20 μM) for indicated periods of time (upper panel). SK-N-AS-Ob-Rb cells were incubated with RuCO (20 μM) for 18h (lower panel), and expression levels of phospho-PEKR, phospho-eIF2α, GRP78 and CHOP were analyzed by western blotting.
(B,C) CHO-K1-Ob-Rb and SK-N-AS-Ob-Rb cells were incubated with TM (2μg/ml) or TG (1μM) for indicated periods of time (upper panel) ; CHO-K1-Ob-Rb and SK-N-AS-Ob-Rb cells were incubated with TM (2μg/ml) or TG (1μM) for 18h (lower panel), and expression levels of phospho-PEKR, phospho- eIF2α GRP78 and CHOP were analyzed by western blotting.

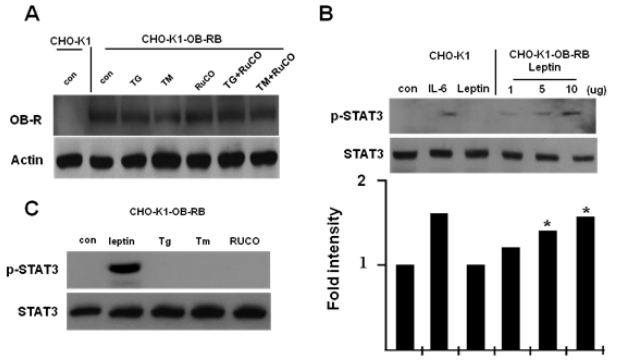

Fig. 2. CO, TG and TM does not affect the expression of OB-R and phosphorylation of STAT3 in CHO-K1-Ob-Rb cells.
(A) CHO-K1-OB-RB cells were treated with TG (1μM), TM (2 μg/ml), RuCO (20 μM), TG (1μM) plus RuCO (20 μM) and TM (2 μg/ml) plus RuCO (20 μM) for 4h, and expression levels of Ob-R were analyzed by western blotting.
(B) CHO-K1 cells were with IL-6 (25ng/ml) and leptin (10μg/ml); CHO-K1-Ob-Rb cells were incubated with leptin (1,5,10 μg/ml) for 15min, phospho-STAT3 and STAT3 levels were analyzed by western blotting (upper panel). phospho-STAT3 levels using image analyzing software (lower panel).
(C) CHO-K1-Ob-Rb cells were treated with TG (1μM), TM (2 μg/ml) and RuCO (20μM) for 15min, phospho-STAT3 and STAT3 levels were analyzed by western blotting. Blots shown are representative of 3 independent experiments. Values are means±SD from 3 independent experiments. *P<0.05 with respect to each untreated group

2. CO has no Effects on the Expression of OB-R and Phosphorylation of STAT3

Tyrosine kinase activates the signal transducer and activator of transcription 3 (STAT3). Thus, we examined whether CO could affect the expression of Ob-R and phosphorylation of STAT3 in CHO-K1-Ob-Rb cell. ER stress did not alter the expression level of leptin receptor. And CORM did not affect expression of Ob-R (Fig. 2A). In transfected cell with Ob-Rb leptin receptor, treatment with leptin increased STAT3 phosphorylation in dose-dependent (Fig. 2B). Treatment of IL-6 is positive control of STAT3 phosphorylation. Next, we examined whether CO could alter phosphorylation of STAT3. Treatment of leptin induced phosphorylation of STAT3, indicating that the leptin signal is activated. Only treatment of CORMhas no effect on phosphorylation of STAT3 as well as ER stress inducer, TM and TG (Fig. 2C). These results suggest that CO has no effect on the expression of OB-R and phosphorylation

3. CO Reverses ER Stress-Induced Leptin Resistance

Because we confirmed that leptin increased STAT3 phosphorylation in CHO-K1 cell transfected with Ob-Rb leptin receptor, we next investigated if CO could block the leptin resistance which is induced by UPR during ER stress in SK-N-AS-Ob-Rb cell. Chemical chaperones such as 4-phenylbutyric acid (4-PBA) also block the ER stress-induced leptin resistance, so leptin-induced STAT3 phosphorylationis recovered. Treatment of CORM reverses ER stress-induced leptin resistance. The STAT3 phosphorylation by leptin was inhibited by ER stress (Fig. 3A). But CORM significantly reversed ER stress-induced leptin resistance in dose-dependent (Fig. 3B). These results suggest that CO reverses ER stress-induced leptin resistance.

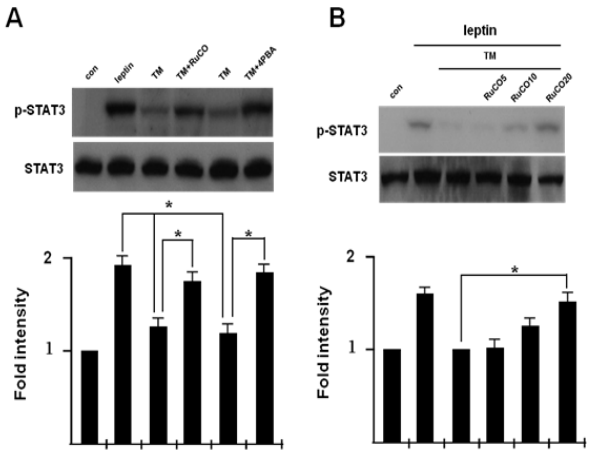

Fig.3. CO reverses ER stress-induced leptin resistance in SK-N-AS-Ob-Rb cells.
(A) SK-N-AS-Ob-Rb cells were preincubated with 4-PBA (5mM) or RuCO (20 μM) for 2h, then treated with TM (2 μg/ml,4h), and then stimulated with leptin (10 μg/ml) for 15 min.
(B) SK-N-AS-Ob-Rb cells were preincubated with RuCO (5,10,20 μM) for 2h, then treated with TM (2 μg/ml,4h), and then stimulated with leptin (10 μg/ml) for 15 min. phospho-STAT3 and STAT3 levels were analyzed by western blotting (upper panel), Densitometric analysis phospho-STAT3 levels using image analyzing software (lower panel). Blots shown are representative of 3 independent experiments. Values are means±SD from 3 independent experiments. *P<0.05 with respect to each untreated group

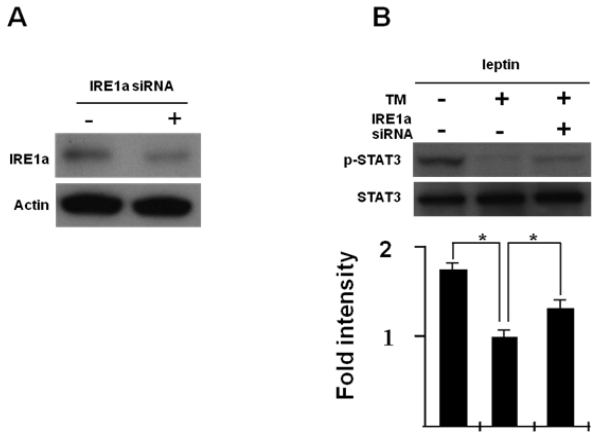

Fig. 4. IRE1 is involved in ER stress-induced leptin resistance in SK-N-AS-Ob-Rb Cells. A and B, SK-N-AS-Ob-Rb cells were transfected with IRE1 siRNA (50nM).
(A) 36h after transfection, Western blotting analysis was performed for IRE1 protein expression.
(B) 36h after transfection, cells treated with TM (2 μg/ml, 4h) and stimulated with leptin (10μg/ml) for 15min were performed Western blotting analysis for phosphor-STAT3 and STAT3 expression. Densitometric analysis of STAT3 phosphorylation was done using image analyzing software. Blots shown are representative of 3 independent experiments. Values are means±SD from 3 independent experiments. *P<0.05 with respect to each untreated group

4. IRE1 is Involved in ER Stress-Induced Leptin Resistance

We observed that CO as well as chemical chaperones, 4-PBA reversed ER stress-induced leptin resistance. Thus, we next investigated whether blocking effects of CO on ER stress-induced leptin resistance might be involved in another ER stress branch, IRE1 in SK-N-AS-Ob-Rb cell. To further determine involvement of IRE1 in ER stress-induced leptin resistance, we used siRNA methodology to silence the IRE1. The siRNA specific for IRE1 was transiently transfected into SK-N-AS-Ob-Rb cells (Fig. 4A). In non-transfected cell, ER stress-induced leptin resistance was observed, but ER stress-induced leptin resistance was decreased in SK-N-AS-Ob-Rb cell transfected with siRNA of IRE1 (Fig. 4B) these results suggest that IRE1 might be involved in ER stress-induced leptin resistance.

5. CO Recovers the ER Stress-Induced Inhibition of Leptin Signaling via Phosphorylation of PERK

Having shown that CO induced phosphorylation of PERK branch of UPRin SK-N-AS-Ob-Rb cells, we further investigated the blockingmechanisms of CO on leptin resistancewhich could be induced by ER stress. Treatment of the cells with CORM increased phosphorylation of PERK and eIF2 ven more than ER stress-induced phosphorylation, but leptin had no effects. These results suggest that CO recovers the ER stress-induced inhibition of leptin signaling via phosphorylation of PERK branch of UPR.

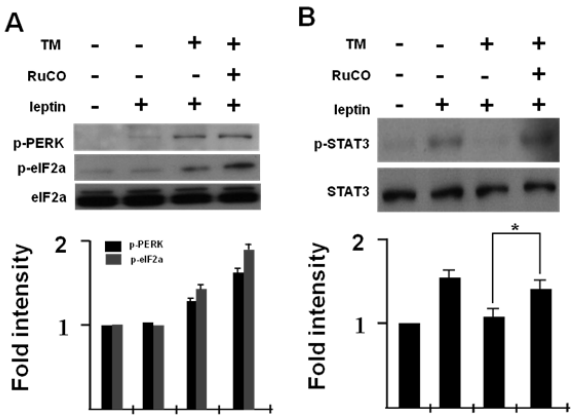

Fig. 5. CO recovers the ER stress-induced inhibition of leptin singnaling in SK-N-AS-Ob-Rb cells via phosphorylation of PERK.
(A,B) SK-N-AS-Ob-Rb cells preincubated with RuCO (20 μM) for 2h, treated with TM (2 μg/ml, 4h), and stimulated with leptin (10 μg/ml) for 15 min were analyzed for phospho-PEKR, PERK, phosph- eIF2α, eIF2α, phospho-STAT3, STAT3 expression by Western blotting (upper panel). Densitometric analysis of phospho-PEKR, phosph- eIF2α, phospho-STAT3 levels using image analyzing software (lower panel). Blots shown are representative of 3 independent experiments. Values are means±SD from 3 independent experiments. *P<0.05 with respect to each untreated group

Ⅳ. DISCUSSION

In the present study, through investigating STAT3 phosphorylation as an indicator of the leptin signal, we have shown that CORM reverses leptin resistance induced by ER stress. Recently Hosoi and Ozcan et al. showed that ER stress contributes to leptin resistance.[11,22] Also it is reported that 4-phenylbutyric acid (4-PBA), a chemical chaperones that can stabilize the protein conformationand improve the protein folding capacity of ER, blocks the ER stress-induced leptin resistance.

We focused on effects of CO on ER stress-induced leptin resistance. We found that CO induced phosphorylation of PERK branch of UPR, but not GRP78 and CHOP. Thus, it is suggested that CO might affect to ER stress-induced leptin resistance through activation of PERK. There is a report that leptin phosphorylates STAT3 protein through the Ob-Rb long isoform of the leptin receptor but not through the short isoform.[23-25] On the other hand, leptin phosphorylates the mitogen-activated protein kinases through the short and long isoforms.[8] The fact that db/db mice (which lack the functional Ob-Rb long isoform of leptin receptor) become obese by increases in food intake and body weight demonstrates the essential role of the Ob-Rb-STAT3 signal for inhibiting food intake and body weight gain for the effect of leptin. Moreover, Bates et al. showed that the replacement of Try1138 in Ob-Rb with a serine residue (ser 1138), which specifically disrupts the Ob-Rb-STAT3 signal, results in marked obesity.[5] These findings indicate that the leptin-induced STAT3 signal is important in reducing obesity. Because leptin receptor Ob-Rb is important for leptin signal, we investigated whether CO could affect on the expression of leptin receptor. CORM had no effects on the expression of leptin receptor. We observed that CORM alone did not alter STAT3 phosphorylation of leptin signal in the cells transfected with Ob-Rb leptin receptors. CO activated Nrf2 through the phosphorylation of PERK, resulting in cytoprotective HO-1 expression.[21] We hypothesized that CO might block the leptin resistance which is induced by UPR during ER stress. Treatment of CORM reverses ER stress-induced leptin resistance. Next, we investigated whether another branch of UPR, IRE1a is involved in ER stress-induced leptin resistance. Because siRNA against IRE1a reverses ER stress-induced leptin resistance and maintains leptin-induced STAT3 phosphorylation and CO induces the phosphorylation of PERK branch of UPR, IRE1a and PERK signals among UPR can alter a status of resistance and susceptibility of leptin signal respectively.
Also these results suggest that IRE1a may be involved in leptin resistance. To further determine the blocking mechanisms of CO on leptin resistance which is induced by UPR of ER stress response, we detailed the effects of CO on PERK branch. CO increased phosphorylation of PERK and eIF2a even more than TM-induced phosphorylation, but leptin had no effects. These results suggest that CO recovers the ER stress-induced inhibition of leptin signaling via phosphorylation of PERK branch. We cannot rule out the possibility that other mechanisms are also involved in CO-induced blocking of leptin resistance. Determining the other mechanisms involved in CO are also subject for

further investigation.

V.CONCLUSION

We have described that CO-induced phosphorylation of PERK branch reversed ER stress-induced leptin resistance. Together, these findings suggest that the pathological mechanism of leptin resistance could be ameliorated by the use of CO donors. The further understanding of ER stress-induced leptin resistance may be critical to clarify the molecular mechanisms/appropriate pharmacological treatment of obesity. Only such chemical chaperones as PBA and tauroursodeoxycholic acid (TUDCA) were recently reported as leptin-sensitizing agents despite the long-standing efforts in both academia and industry.[11] The results presented in this study provide evidence that one of bio-gases CO can be used as leptin-sensitizing agents when the leptin resistance is caused by ER stress. When the high safety profiles of CORM, CO inducers or CO gas itself takes count into consideration, leptin could be used as a novel therapeutic option for obesity.

국문초록

연구 배경 및 목적: 렙틴이 음식의 섭취 양과 체중의 증가를 억제하는 중요한 호르몬 역할을 한다는 사실은 잘 알려졌다. 또한 렙틴에 대한 저항성이 비만의 중요한 원인이라는 연구가 많이 보고되고 있다. 그러나 렙틴 저항성에 대한 구체적인 기전은 아직 밝혀져 있지 않았다. 최근 endoplasmic reticulum (ER) 스트레스에 의한 unfolded protein response (UPR) 가 렙틴에 대한 저항성을 유발한다는 보고가 있었다.

연구 대상 및 방법: 일산화탄소(carbon monoxide, CO)는 heme oxygenase (HO)-1이 헴(heme)을 산화적 분해로 만들어내는 무색, 무미, 무취의 기체로 항산화, 항염증, 항세포분열 및 항세포고사 작용을 나타내며 조직의 손상에 대한 세포보호작용을 한다. 최근 CO는 ER stress sensor 중의 하나인 protein kinase R-like endoplasmic reticulum kinase (PERK) 를 선택적으로 활성화 시키며 인산화된 PERK 는 HO-1의 전사조절인자인 Nrf2를 인산화로 활성화시켜 결국 HO-1의 유전자 발현을 촉진함이 보고 되였다. 또한 CO로 유도된 PERK의 활성화는 eukaryotic translation initiation factor (eIF) 2a를 인산화로 활성화 시키고 인산화된 eIF2a는 전사인자인 activating transcription factor (ATF)-4의 발현을 유도하지만 CO는 ER stress의 다른 두 개 sensors인 inositol requiring enzyme (IRE) 1a와 하위신호전단물질인 X-box binding protein (xbp)-1이나 ATF6의 활성화에는 영향을 미치지 아니한다. 그러나 ER stress 유발인자들인 thasigargin (TG), tunicamycin (TM), homocysteine 등으로 Xbp-1이나 ATF6가 활성화되는 것을 억제한다.

연구 결과: 본 연구에서는 ER stress 에 의해 활성화되는 UPR에 의해 유발되는 렙틴 저항성이 CO에 의하여 차단될 것이라는 가정을 세우고 다음과 같은 실험결과를 얻었다. TG나 TM에 의해 유도된 ER stress반응 즉 UPR은 렙틴의 신호전달로 야기되는 phospho-signal transducer and activation of transcription (STAT) 3로 인산화되는것을 억제시켰다. 그러나 ER stress를 유도시킬 시 CO를 CO releasing molecule (CORM)으로 처리하였을 때 UPR에 의한 렙틴의 신호전달로 말미암는 STAT3 인산화의 차단이 CO의 양과 노출 시간에 비례하여 제거 되었다.
결론: 이러한 실험결과는 CO에 의한 PERK 인산화는 ER stress 유도체에 의한 IRE1a의 하위 신호전달물질인 Xbp-1 이나 ATF6의 활성화를 억제하여 ER stress 반응인 UPR에 의한 렙틴 저항성을 차단함을 시사한다. 결론적으로 이러한 실험결과는 CO유도체를 이용하여 병인적인 렙틴 저항에 의한 여러 질병을 치료할 수 있음을 시사한다.

중심 단어: Endoplasmic reticulum (ER), Stress, Unfolded protein response(UPR), Carbonmonoxide(CO), Leptin resistance, phospho-signal transducer and activation of transcription3 (STAT3), Leptin

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