Mycophenolic acid inhibits oleic acid-induced mesangial cell activation through both cellular reactive oxygen species and inosine monophosphate dehydrogenase 2 pathways

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Directed by Professor Soon Il Kim

The Doctoral Dissertation submitted to the Department of Medicine, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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ABSTRACT

Mycophenolic acid inhibits oleic acid-induced mesangial cell activation through both cellular ROS and inosine monophosphate dehydrogenase 2 pathways

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(Directed by Professor Kim Soon Il)

Extracellular matrix (ECM) synthesis in mesangial cells (MCs) plays important roles in the development and the progression of renal diseases including chronic allograft nephropathy. Mycophenolic acid (MPA), an inhibitor of inosine monophosphate dehydrogenase 2 (IMPDH2), suppressed MC proliferation and ECM synthesis. However, the exact inhibitory mechanism of MPA on MCs has not been clearly

elucidated. In this study, we compared the inhibitory effects of MPA and IMPDH2 reduction (by using siRNA) on OA-induced fibronectin secretion and cellular ROS in mouse MCs. Growth-arrested MCs were stimulated with OA in the presence or absence of MPA, IMPDH2 siRNA, N-acetylcystein (NAC) or transforming growth factor (TGF)-β antibody or exogenous guanosine. Fibronectin secretion into the medium was examined by Western blot, DCF-sensitive cellular ROS by FACS, TGF-β levels in the media by ELISA.

OA increased fibronectin secretion, TGF-β and cellular ROS levels. A TGF-β neutralizing antibody effectively suppressed OA-induced fibronectin secretion. NAC and MPA completely suppressed OA-induced fibronectin secretion, and decreased the levels of TGF-β and cellular ROS. However, IMPDH2 siRNA partly inhibited OA-induced MMC activation. Exogenous guanosine successfully reversed the inhibitory effects of IMPDH2 siRNA on OA-induced MC activation.

Pleiotropic inhibitory effect of MPA on OA-induced mouse MC activation was mediated via its antioxidant effect on cellular ROS production and partly via inhibition of IMPDH2 itself. Our results

implicate ROS as an alternative therapeutic target for the prevention of hyperlipidemia-related glomerulopathy, chronic allograft nephropathy, and subsequent graft loss.

Key words: mycophenolic acid, chronic allograft nephropathy, mesangial cell, inosine monophosphate dehydrogenase 2, oleic acid, hyperlipidemia, extracellular matrix, reactive oxygen species

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I. INTRODUCTION

Chronic allograft nephropathy (CAN) is the most common cause of late graft failure in renal transplant recipients; both immunological and non-immunological factors contribute to the development of CAN [1-5]. CAN most commonly presents with vasculopathy, glomerulosclerosis, interstitial fibrosis, and tubular atrophy [1, 3, 6, 7]. Renal mesangial cells (MCs) are located in the pericapillary mesangial space of the glomerular capillary tuft, and they are classified as specialized smooth muscle cells [8]. MCs participate in the inflammatory glomerular disease process by increasing growth factor

secretion, cell proliferation, and extracellular matrix (ECM) deposition, all of which contribute to glomerular damage [9]. MC proliferation and ECM accumulation typically characterize glomerulosclerosis; ECM accumulation is also considered an etiological hallmark of CAN [2, 4, 5].

Hyperlipidemia, one of the common metabolic complications of renal transplantation, develops in at least 60% of recipients as a side effect of immunosuppressive therapy [10-12]. Hyperlipidemia not only increases the incidence of cardiovascular disease, but it also accelerates the progression of CAN following transplantation [13-15]. Hyperlipidemia-induced CAN progression is mediated in part by oleic acid (OA, an 18-carbon lipid with one *cis* double bond [18:1]), an abundant fatty acid in plasma triglycerides, that promotes the proliferation of vascular smooth muscle cells (VSMCs) in rat [16, 17] and human [18]. However, the mechanism of induced CAN progression is not yet clear.

Reactive oxygen species (ROS) have recently been proposed as important signaling molecules in many biological events, including cell proliferation, cell death, and gene expression [19-22]. Many previous reports suggested that cellular ROS might be a major second messenger in the ECM synthesis. Furthermore, OA-induced VSMC proliferation is also mediated by ROS. However, the role of ROS in ECM synthesis of renal MCs is not clear.

Mycophenolic acid (MPA), an inhibitor of inosine monophosphate dehydrogenase 2 (IMPDH2), inhibits not only lymphocyte, but also mesenchymal cell proliferation [23-28]. We also reported that MPA inhibited the VSMC and MC proliferation and ECM synthesis [26]. However, IMPDH2 is only partially involved in the inhibitory action of MPA on mesenchymal cells [29]. Other possible downstream MPA targets include ROS. The anti-oxidative effect of MPA is not yet reported in MCs.

Herein, to elucidate the mechanisms on OA-induced mesangial ECM synthesis and the effects of MPA on OA-induced mouse MC activation, we investigated the effects of OA on mesangial secretion of fibronectin and TGF-β into the media, and cellular ROS levels, and compared the different inhibitory effects of MPA and IMPDH2 siRNA in these OA-induced molecular events.

II. MATERIAL AND METHODS

1. Mouse mesangial cell culture

Mouse MCs derived from an SV40 transgenic mouse (MES-13) were purchased from American Type Culture Collection (Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Gaithersberg, MD, USA) supplemented with 5% fetal bovine serum (FBS, Invitrogen). MCs were maintained and incubated at 37°C in humidified air containing 5% CO_2. All experiments were conducted in serum-free DMEM media. MPA (0.1, 1 and 10 μM), TGF-β neutralizing antibody (25 μg/ml; R&D Systems, Minneapolis, MN, USA) or N-acetylcysteine (NAC, 5 mM) was administered 1 hour before the addition of OA (100 μM). Exogenous guanosine 100 μM was added at the same time with IMDPH2 siRNA transfection. The optimal concentration of NAC or TGF-β neutralizing antibody was determined in preliminary experiments (data not shown).

2. Transfection of IMPDH2 siRNA

Pre-designed and annealed mouse IMPDH2 siRNA was purchased from Ambion (Austin, TX, USA). IMPDH2 siRNA was transfected using Lipofectamine (Invitrogen) for 24 hours, according to the manufacturer’s

instructions.

3. Methylthiazoletetrazolium (MTT) assay for mesangial cell viability

Cell viability was measured by MTT assay as previously described [25]. After drug treatment, 1 mg/ml MTT was added to each well, and the cultures were incubated for 2 hours. Extraction buffer (20% SDS, 50% N,N-dimethylformamide, pH 4.7) was then added. After 24 hours, the amount of formazan product was spectrophotometrically quantified at 562 nm using a microplate reader (SpectraMax 340, Molecular Devices Co., CA, USA).

4. Real time RT-PCR for IMPDH2 mRNA expression

Expression of the mRNAs was assessed in real-time RT-PCR assays using the SYBR green system (ABI PRISM 7700 Sequence Detection System, Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. Briefly, total RNA was extracted from MCs using TRIzol reagent (Invitrogen). Reactions were assembled with 5 μg RNA in 20 μl total reaction volumes (Maxime RT premix, Intron, Seoul, Korea). Reactions were conducted for 50 minutes at 42°C, followed by incubation at 95°C for 10 min to synthesize cDNA. The PCR was performed using the SYBR premix Ex Taq kit (Takara, Otsu, Shiga, Japan) corresponding to either mouse IMPDH2

(Forward; GCTGATGGAGGAATCCAAAA, Reverse; TGGCTGCTGAGATGTTTGTC) or mouse GAPDH (Forward; TGCACCACCAACTGCTTAG, Reverse; GGATGCAGGGATGATGTTC) as a control. Levels of mRNA were quantified using a comparative C_T method and presented as relative expression normalized to GAPDH. To confirm amplification specificity, the PCR products were subjected to a melting curve analysis and subsequent agarose gel electrophoresis.

5. Western blot analysis for fibronectin secretion

Following pharmacological treatment, media were collected and centrifuged to remove cell debris. To measure fibronectin secrtion into the media, calculated media by cell protein concentration were mixed with loading buffer (60 mM Tris-HCl, 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol, 0.1% bromophenol blue), separated by 6% SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). After being blocked for 1 hour with 5% non-fat dry milk, the membranes were then incubated with peroxidase-conjugated polyclonal rabbit anti-human fibronectin (1:5,000, DAKO Japan Co., Tokyo, Japan) bound with primary and secondary antibody. Immunoreactive sites were visualized by an enhanced chemiluminescence (ECL; Amersham, Buckinghamshire, UK)

Western blotting detection system and quantified using a densitometer.

6. Enzyme-linked immunosorbent assay (ELISA) for TGF-β1 secretion

The levels of TGF-β1 secreted into the culture medium under different experimental condition were determined using a commercial TGF-β1 ELISA kit (R&D Systems) sensitive to 4.6 pg/mL, according to the manufacturer's protocol.

7. Flow cytometry for cellular reactive oxygen species (ROS)

After pharmacological treatment, cells were washed twice with PBS and combined with 5 μM 5-(and-6)-chloromethyl-2`,7`-dichlorodihydro-fluorescein diacetate (DCFH-DA; Molecular Probes, Eugene, OR, USA)in PBS. Cultures were incubated at 37°C under 5% CO_2 for 20 minutes. After unbound dye was removed by washing with PBS, fluorescence (excitation: 485 nm, emission: 530 nm) was measured by fluorescence-activated cell scanning (FACS; Becton Dickinson Immunocytometry System, Mountain View, CA, USA).

8. Statistical analysis

Results are expressed as means ± standard error of five experiments, and statistical comparisons were evaluated by ANOVA followed by Fisher's least

significant difference method. Differences were deemed significant if $P < 0.05$.

III. RESULTS

1. OA-induced fibronectin secretion was mediated by TGF-β.

To determine the working concentration of OA for MCs, we conducted a dose-response assay. We treated cells with increasing concentrations of OA [0, 50, 100, 200 μM, or ethanol (EtOH) alone] and measured the effects on fibronectin secretion and TGF-β1 levels in the media at 24 hours after the addition of OA. Fibronectin secretion by 100 μM OA was peaked and that was 1.8-fold compared to control (Fig. 1A).

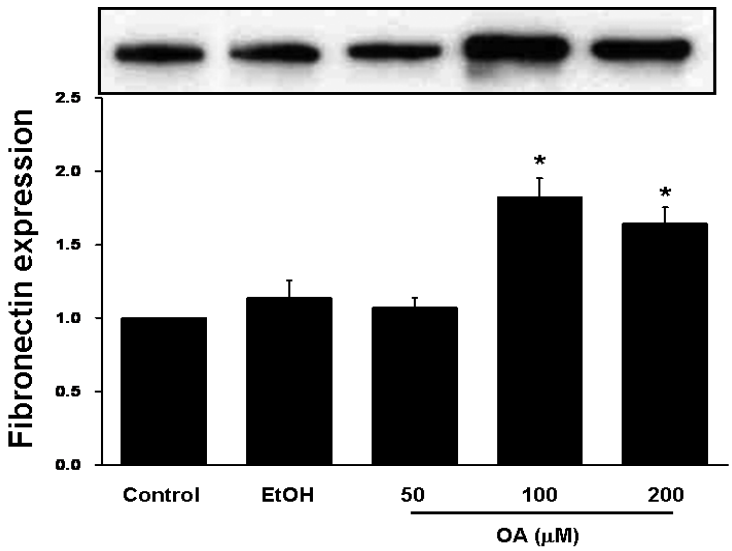

Figure 1A. Dose-response effect of oleic acid (OA) on fibronectin secretion into the media. *P <0.05 vs. control.

OA at 100 μM also maximally increased TGF-β1 levels compared to control (772 ± 59 vs. 1,459 ± 57 pg/ml, $p<0.05$, Fig. 1B). Since TGF-β1 is the most important profibrogenic cytokine, we sought to elucidate whether it mediated OA-induced fibronectin secretion. Treatment with a TGF-β neutralizing antibody significantly suppressed OA-induced fibronectin secretion (Fig. 1C).

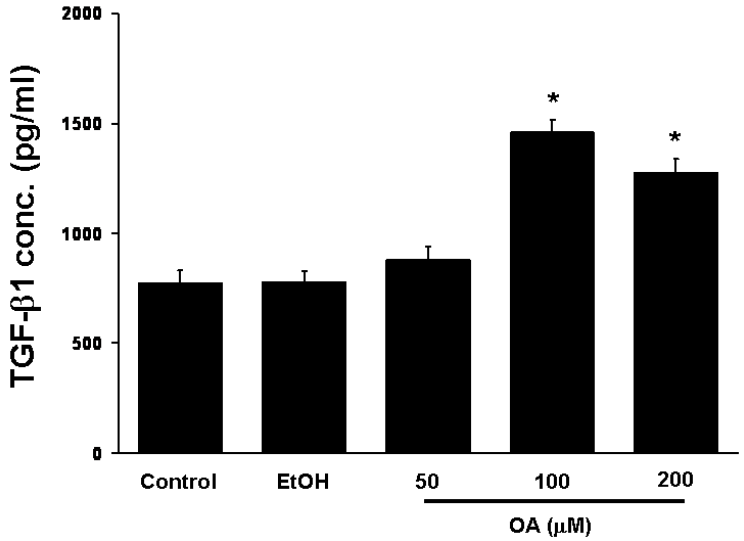

Figure 1B. Dose-response effect of OA on TGF-β1 levels in the media.
*P <0.05 vs. control.

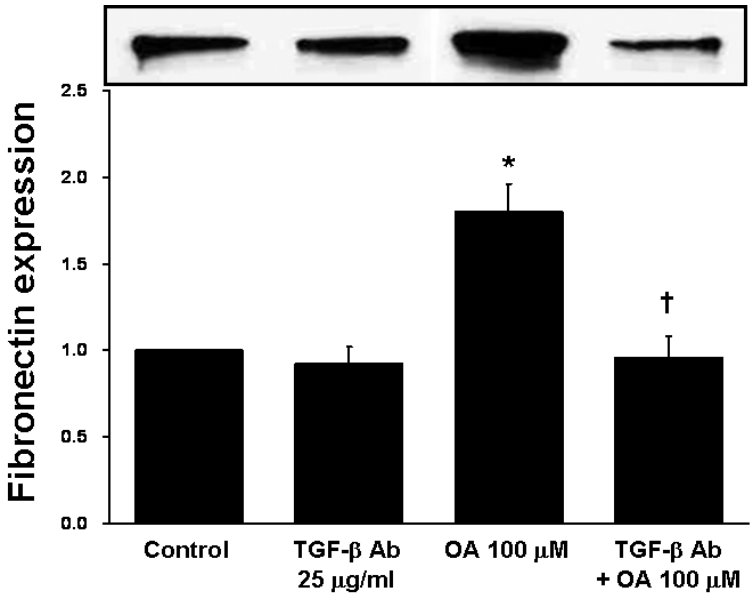

Figure 1C. Effect of TGF-β neutralizing antibody (Ab) on OA-induced fibronectin secretion. *P <0.05 vs. control. †P < 0.05 vs. OA (100 μM) treatment alone.

2. Treatment of OA significantly increased cellular ROS

Treatment of 100 μM OA increased cellular ROS by 2.0-fold, starting 5 minutes after OA addition and lasting for at least 60 minutes. Treatment with the antioxidant NAC (5 mM) effectively inhibited OA-induced cellular ROS levels at 5 minutes after OA addition (Fig. 2A).

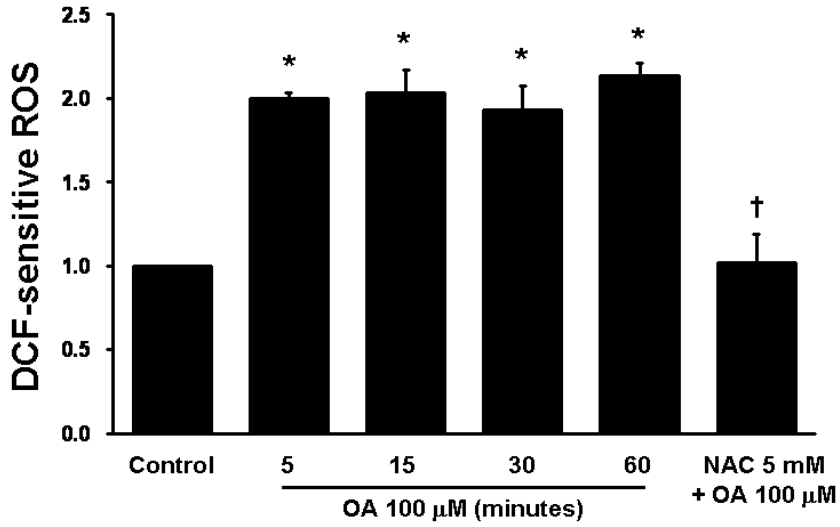

Figure 2A. Time course of OA-induced cellular ROS. *P <0.05 vs. control. †P < 0.05 vs. OA (100 μM) treatment alone.

To examine the role of cellular ROS on OA-induced fibronectin secretion and TGF-β1 levels in the media, we added 5 mM NAC 1 hour before the addition of OA. NAC significantly ameliorated OA-induced fibronectin secretion (Fig. 2B) and TGF-β1 levels in the media (Fig. 2C).

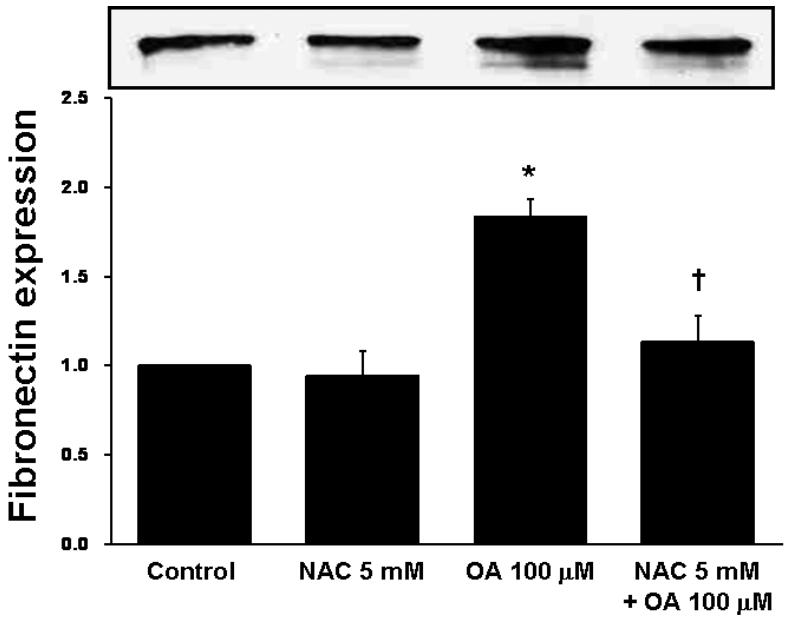

Figure 2B. Effect of NAC on OA-induced fibronectin secretion into the media. *P <0.05 vs. control. †P < 0.05 vs. OA (100 μM) treatment alone.

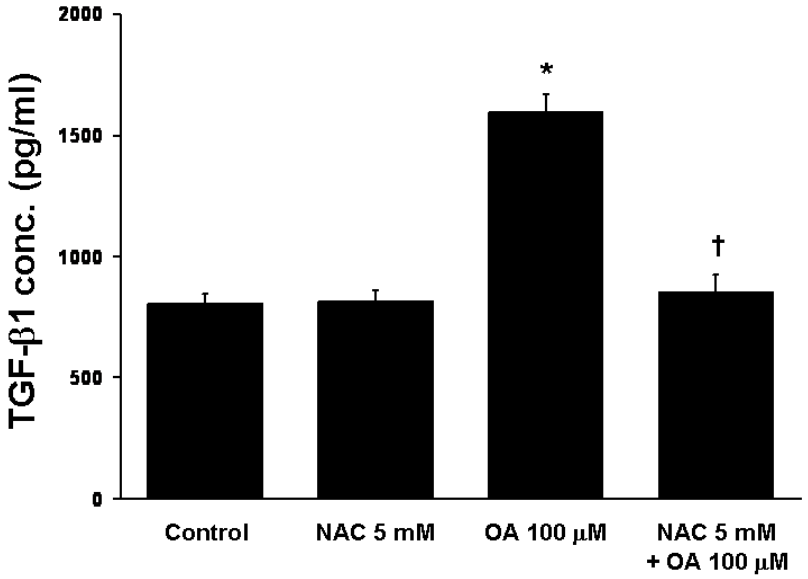

Figure 2C. Effect of NAC on OA-induced TGF-β1 levels in the media. *P <0.05 vs. control. †P < 0.05 vs. OA (100 μM) treatment alone.

3. MPA completely inhibited OA-induced MC activation

MPA at above 1 μM completely inhibited the OA-induced fibronectin secretion (Fig. 3A) and decreased TGF-β1 levels in the media (Fig. 3B) without affecting basal values. But, OA-induced cellular ROS was abrogated by MPA above 0.1 μM, a relatively lower concentration (Fig. 3C).

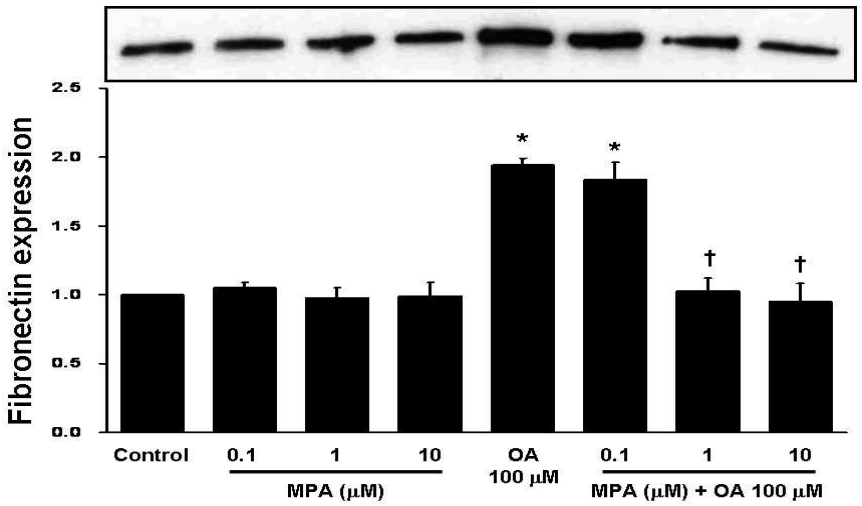

Figure 3A. Effect of MPA on OA-induced fibronectin secretion into the media. *P < 0.05 vs. control. †P < 0.05 vs. OA (100 μM) treatment alone.

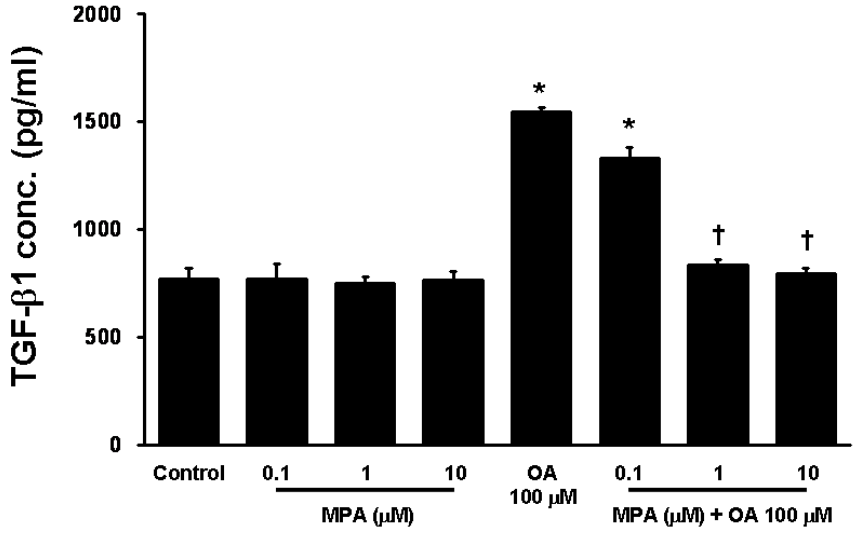

Figure 3B. Effect of MPA on OA-induced TGF-β1 levels in the media.

*P < 0.05 vs. control. †P < 0.05 vs. OA (100 μM) treatment alone.

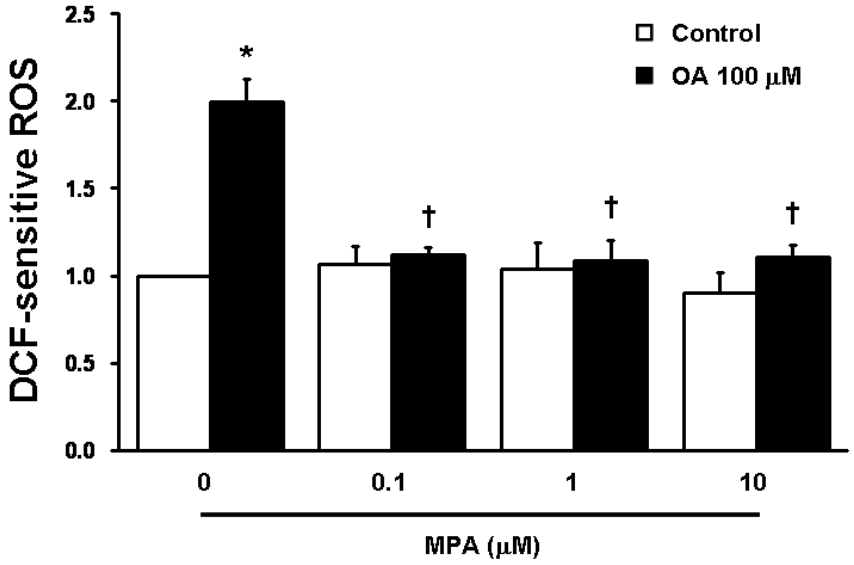

Figure 3C. Effect of MPA on OA-induced cellular ROS. *P < 0.05 vs. control. †P < 0.05 vs. OA (100 μM) treatment alone.

4. IMPDH2 siRNA partly reduced OA-induced MC activation

The efficacy of IMPDH2 siRNA transfection was evaluated by IMPDH2 mRNA expression level. IMPDH2 mRNA expression was effectively decreased by IMPDH2 siRNA-transfected MCs in a dose-dependent manner (Fig. 4A).

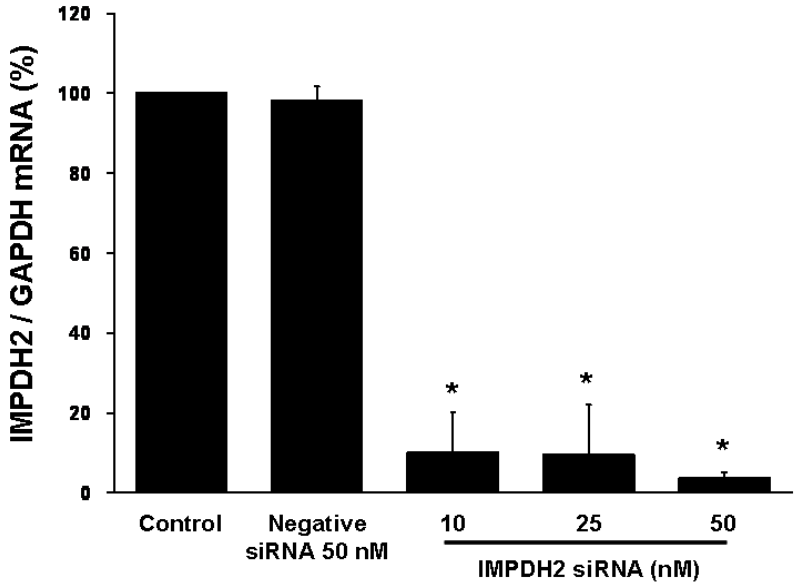

Figure 4A. Effect of IMPDH2 siRNA on IMPDH2 mRNA expression. *P < 0.05 vs. control.

In contrast, negative control of IMPDH2 siRNA had no effects on IMPDH2 mRNA expression. Introduction of up to 50 nM IMPDH2 siRNA also significantly ameliorated OA-induced fibronectin secretion (Fig. 4B) and TGF-β1 levels in the meida (Fig. 4C), but the final levels were still 1.4-fold of control (for fibronectin) and 1,029 ± 48 pg/ml (for TGF-β1), respectively. Cellular ROS levels were inhibited partly by 1.5-fold of control (Fig. 4D).

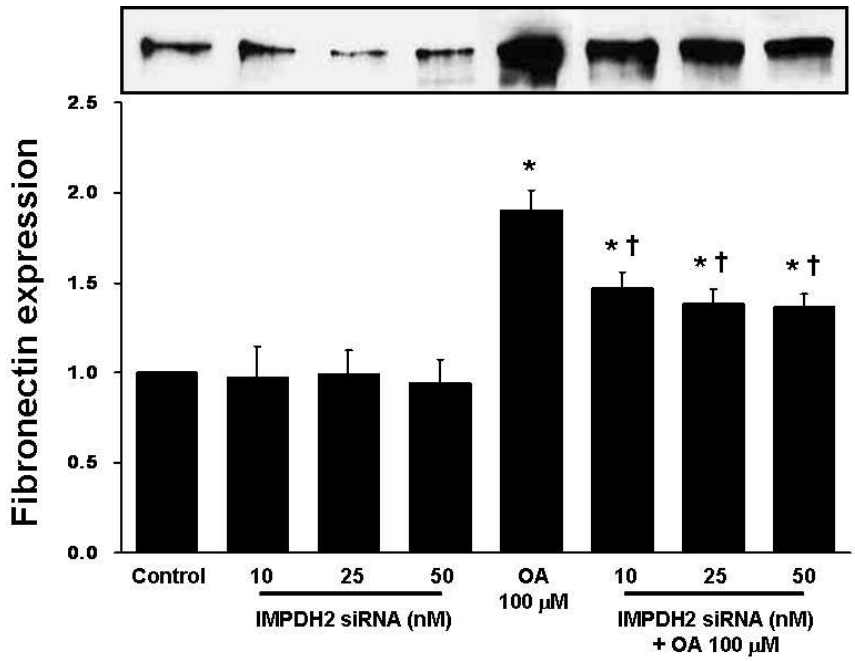

Figure 4B. Effect of IMPDH2 siRNA on OA-induced fibronectin secretion into the media. *P < 0.05 vs. control. †P < 0.05 vs. OA (100 μM) treatment alone.

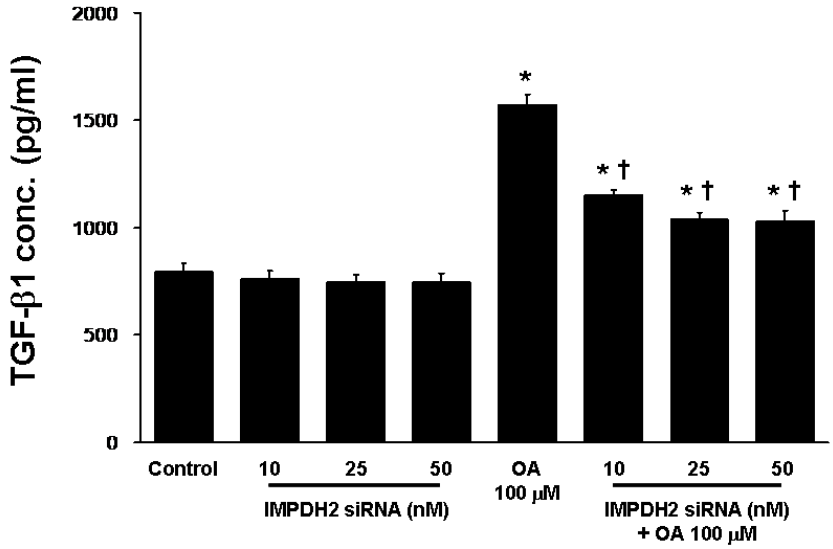

Figure 4C. Effect of IMPDH2 siRNA on OA-induced TGF-β1 levels in the media. *P < 0.05 vs. control. †P < 0.05 vs. OA (100 μM) treatment alone.

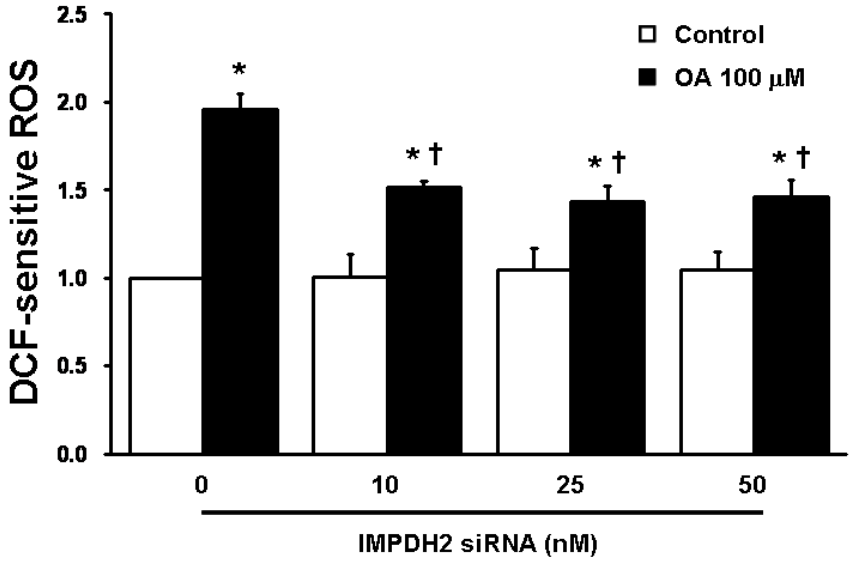

Figure 4D. Effect of IMPDH2 siRNA on cellular ROS. *P < 0.05 vs. control. †P < 0.05 vs. OA (100 μM) treatment alone.

5. Exogenous guanosine completely reversed the inhibitory effects of IMPDH2 siRNA on OA-induced MC activation.

Addition of exogenous guanosine 100 μM did not affect OA-induced MC activation. Levels of fibronectin (Fig. 5A), TGF-β1 (Fig. 5B) and cellular ROS (Fig. 5C) were partly reduced by IMPDH2 siRNA. They were completely restored to basal levels (OA-stimulated control) by addition of exogenous guanosine.

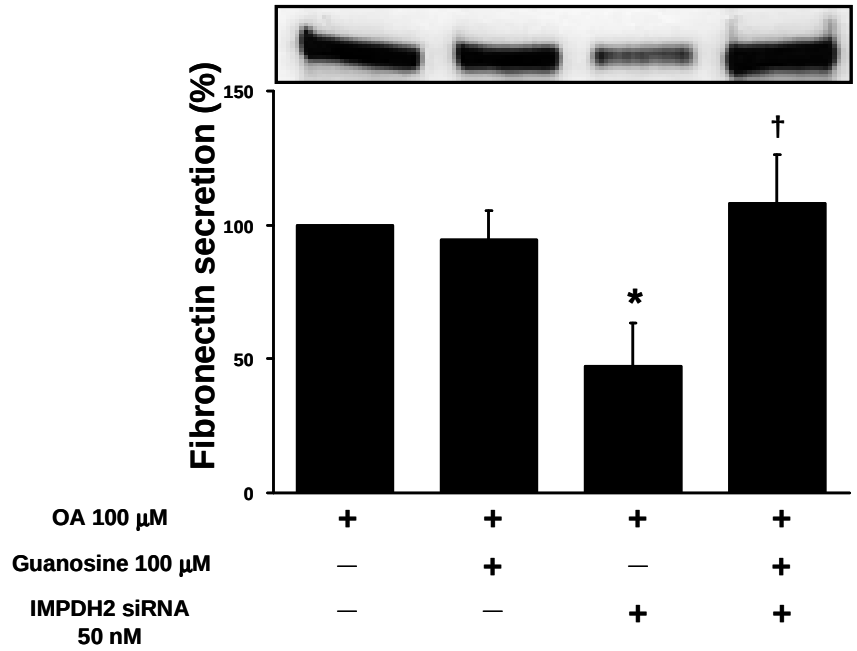

Figure 5A. Effect of guanosine on fibronectin secretion into the media. *P < 0.05 vs. OA (100 μM) treatment alone. †P < 0.05 vs. OA and IMPDH2 siRNA..

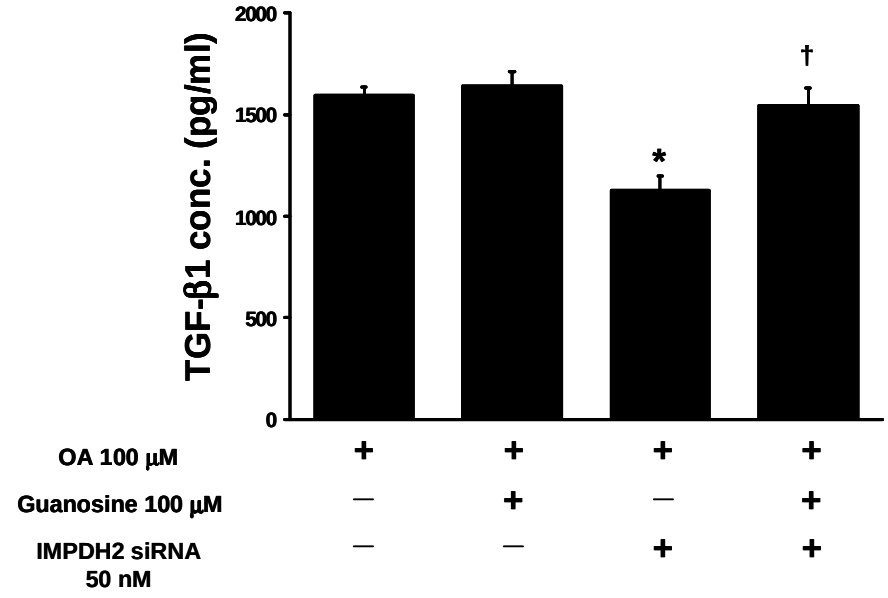

Figure 5B. Effect of guanosine on TGF-β1 levels in the media. *P < 0.05 vs. OA (100 μM) treatment alone. †P < 0.05 vs. OA and IMPDH2 siRNA.

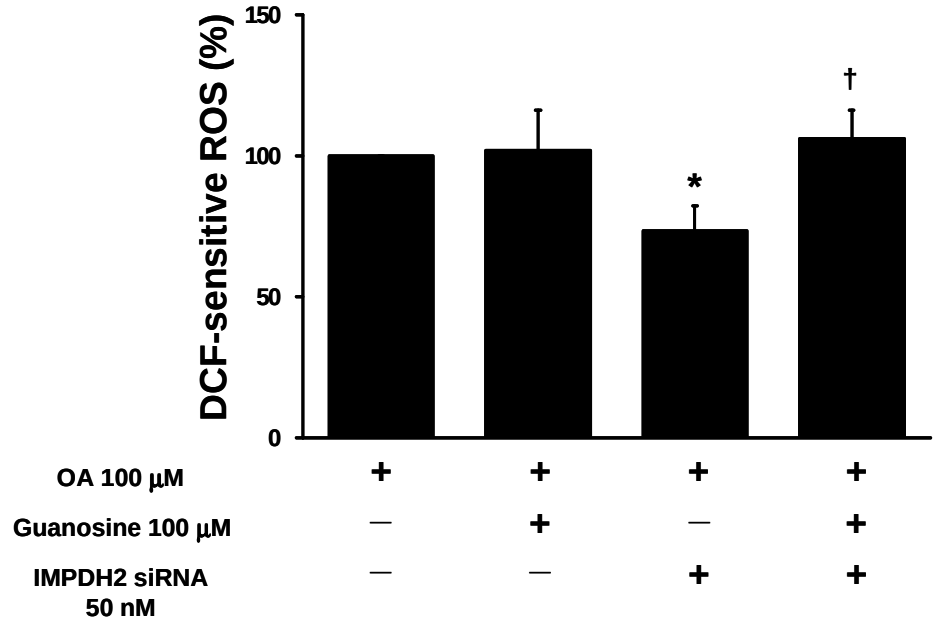

Figure 5C. Effect of guanosine on cellular ROS. *P < 0.05 vs. OA (100 μM) treatment alone. †P < 0.05 vs. OA and IMPDH2 siRNA.

6. MPA and IMPDH2 siRNA did not affect basal viability of MCs

The concentrations of IMPDH2 siRNA and MPA used in this study did not affect MC viability (Fig. 6).

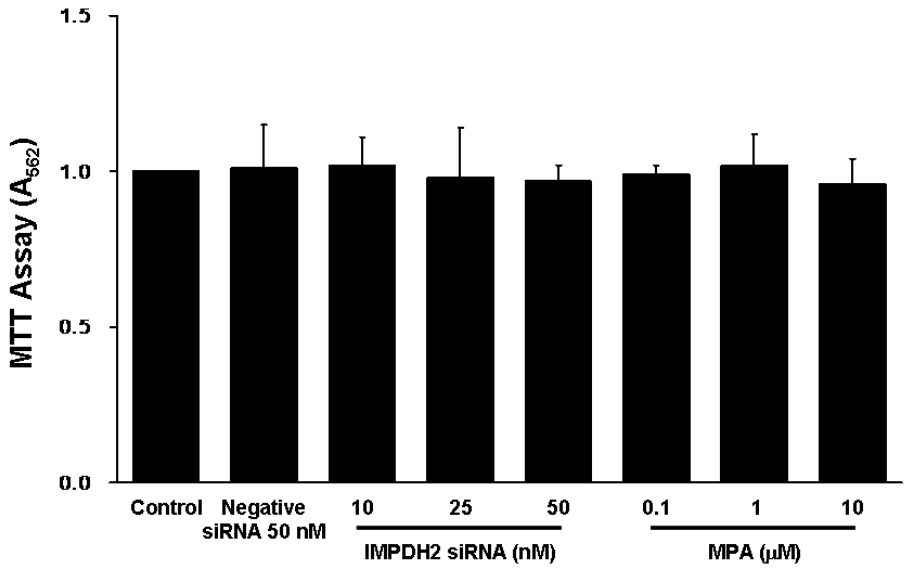

Figure 6. Effect of MPA and IMPDH2 siRNA on MC viability.

IV. DISCUSSION

Numerous studies have suggested that free fatty acids play important roles for the development and progression of atherosclerosis and glomerulosclerosis including CAN [1-5]. Although there are many reports about OA-induced VSMC activation and related cellular mechanisms, OA-induced MC activation has been scarcely reported. In this study, we demonstrate for the first time that OA itself may increase fibronectin secretion through TGF-β1 and cellular ROS in MC. In addition, the results of the present study show that MPA inhibit OA-induced MC activation partly, but not completely, through IMPDH2 pathway.

Fibronectin secretion and TGF-β1 levels in the media were increased in OA-stimulated MCs and a TGF-β neutralizing antibody completely blocked OA-induced fibronectin secretion. These results were in accordance with the results of the previous study, which OA-albumine complexes induces myofibroblast-like phenotype in MCs [30].

To reveal the signaling pathway, we examined the role of cellular ROS in OA-treated MCs. OA increased cellular ROS at 5 minutes in MCs, and addition of NAC at 5 mM significantly inhibited OA-induced fibronectin secretion and TGF-β1 synthesis as well as cellular ROS. These results agreed with the previous results in VSMCs [17]. With these results, we could confirm

that cellular ROS is also involved in OA-induced activation of MCs.

A previous report identified guanosine depletion as a possible mechanism behind MPA-mediated inhibition of MCs [31]. However, addition of exogenous guanosine only partially restored MPA-mediated inhibition of VSMC proliferation, suggesting that there are other mechanisms at work in addition to guanosine depletion [25]. Considering that the role of IMPDH in guanosine synthesis is limited at best in MCs, herein we considered other possible mechanisms that might contribute to the inhibitory effect of MPA. Supporting this concept is our previous observation that MPA inhibited PDGF-induced MC proliferation and ECM synthesis partly by inhibiting p38 MAPK activation [26].

MPA significantly inhibited OA-induced secretion of fibronectin and TGF-β1 into the media. These results are consistent with those of earlier studies, in which MPA inhibited PDGF- or TGF-β1 induced fibronectin synthesis in rat [26] and human [28] MCs, respectively. Furthermore, MPA can prevent the CAN progression through inhibiting TGF-β and smad pathway [32]. The concentrations of MPA (1-10 μM) being required to inhibit OA-induced secretion fibronectin and TGF-β1 is clinically attainable [29, 33]. Since MPA mediates its effects at least in part by inhibiting IMPDH2, we investigated whether IMPDH2 reduction (*via* siRNA) replicated the effects of MPA.

Previous studies have used exogenous guanosine administration to show the role of IMPDH2, but it may influence all guanosine from other sources as well as IMPDH2. Thus, we tried to inhibit IMPDH2 by using specific siRNA. IMPDH2 siRNA (≥10 nM) partly, but not completely suppressed OA-induced secretion of fibronectin and TGF-β1. Although guanosine supplementary could completely counterbalance the inhibitory effects of IMPDH2 siRNA on OA-induced MMC activation, role of IMPDH2 as the underlying mechanism of MPA action should be limited because IMPDH2 siRNA only partly blocked OA-induced cell activation. These results suggest the presence of IMPDH2-independent antioxidant mechanism within MPA.

As already reported, transient siRNA transfection may not wholly block the activity of IMPDH2. Although IMPDH2 siRNA inhibited 90% of IMPDH2 mRNA expression, the remained small amount of enzyme may be sufficient for the target gene expression. In our view, the IMPDH2 gene silencing actually has merit for linking MPA more closely to the function of this gene due to these limitations. Limitations of IMPDH2 antibody and activity measurement may raise the argument. If the correlation of mRNA and protein is not closely matched, partial reduction of MC activation by IMPDH2 siRNA may not be fully understood. Despite of these controversial points, our findings indicated that MPA may have broader, pleiotropic effects compared

to IMPDH2 inhibition itself and that other mechanisms may also be responsible for the effects of MPA. As a possible mechanism, we previously suggested that MPA directly or indirectly inhibit PDGF-induced cellular ROS in VSMCs [34]. Our present study demonstrated that MPA also completely inhibited OA-induced cellular ROS in MCs, whereas IMPDH2 siRNA partly suppressed OA-induced cellular ROS. These results suggest that IMPDH2 is one of the important mediators for OA-induced cellular ROS, but that ROS up-regulation is not completely dependent on IMPDH2 in MCs. In the present study, MPA inhibited OA-induced cellular ROS at a lower concentration than that is required for the inhibition of fibronectin secretion and TGF-β1 secretion. These findings suggested that antioxidant effect of MPA may be necessary but not sufficient to inhibit OA-induced ECM synthesis.

Taken as a whole, our observations strongly indicated that MPA inhibit OA-induced fibronectin secretion through inhibition of cellular ROS, in addition to partial inhibition IMPDH2 itself. These findings corroborates previous studies in which MPA inhibited PDGF or OA-induced VSMC proliferation and ECM synthesis through inhibiting cellular ROS [17, 25]. Recently, MPA inhibition of Rac1-dependent endothelial NAD(P)H oxidase activity and subsequent superoxide formation was reported [35].

V. CONCLUSION

OA-induced fibronectin secretion is mediated by TGF-β and cellular ROS, and MPA inhibits OA-induced MC activation through both cellular ROS and IMPDH2 pathways. Our results implicate ROS as a possible therapeutic target for the prevention of hyperlipidemia-related transplant glomerulopathy, CAN, and subsequent graft loss.

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ABSTRACT (in Korean)

Mycophenolic acid의 세포내 활성산소족과 inosine monophosphate dehydrogenase 2 경로를 통한 oleic acid에 의해 유도된 사구체 혈관사이세포의 활성화 억제

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만성이식신병증은 이식후기에 이식신 소실의 가장 흔한 원인이다. 사구체 혈관사이세포에서의 세포외기질 합성은 만성이식신병증의 발생과 진행에 중요한 역할을 하는것으로 알려져있다. 면역억제제로 흔히 사용되고 있는 Mycophenolic acid (MPA)는 세포내 guanosine 합성에 중요한 효소인 inosine monophosphate dehydrogenase2 (IMPDH2)에 대한 선택적이고 비경쟁적인 억제제로서 사구체 혈관사이세포의 증식과 세포외기질의 합성을 억제한다. 그러나, MPA가 사구체 혈관사이세포를 억제하는 정확한 기전은 알려지지 않았다.

이번 연구에서는 MPA와 IMPDH2의 small interference RNA (siRNA)가 oleic acid에 의해 유도된 사구체 혈관사이세포의 fibronectin 분비와 세포내 활성산소족 생성에 미치는 영향을 비교 검색하였다.

Oleic acid로 유도한 생쥐 사구체 혈관사이세포에 MPA, IMPDH2 siRNA, N-acetylcystein (NAC), transforming growth factor (TGF)-β 항체 또는 guanosine을 투여하였다. Fibrocentin 분비는 Western blot, DCF에 민감한 세포내 활성산소족은 fluorescence-activated cell scanning (FACS), 그리고 TGF-β는 enzyme-linked immunosorbent assay (ELISA) 방법을 이용하여 측정하였다.

Oleic acid는 fibronectin 분비 및 세포내 활성산소족 생성을 증가시켰다. TGF-β 항체는 oleic acid에 의한 fibronectin 분비를 효과적으로 감소시켰다. NAC와 MPA는 oleic acid에 의한 fibronectin, TGF-β, 및 세포내 활성산소족 생성을 완전히 억제하였다. 그러나 IMPDH2 siRNA는 oleic acid에 의한 이러한 사구체 혈관사이세포의 활성을 부분적으로 억제시켰다. 외부에서 투여한 guanosine은 IMPDH2 siRNA의

사구체 혈관사이세포의 활성 억제를 완전히 회복시켰다.

본 연구 결과는 MPA의 oleic acid에 의한 사구체 혈관사이세포의 활성화 억제가 세포내 활성산소족에 대한 억제 기전을 경우하여 발생할 수 있고 부분적으로는 IMPDH2 억제 자체에 의한 것임을 시사하였다. 이러한 결과를 바탕으로 임상적으로는 고지혈증에 의한 사구체신병증, 만성이식신병증, 이식신 소실을 예방하기 위하여 활성 산소족이 주요한 치료 표적이 될 수 있다.

핵심되는 말: mycophenolic acid, 만성이식신병증, 사구체 혈관사이세포, inosine monophosphate dehydrogenase2, oleic acid, 고지혈증, 세포외기질, 활성산소족