Interleukin-13 may increase podocyte permeability via modulation of zonula occludens-1

Se Jin Park

Department of Medicine

The Graduate School, Yonsei University

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Directed by Professor Jae Il Shin

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

Se Jin Park

June 2013

This certifies that the Doctoral

Dissertation of Se Jin Park is approved.

Thesis Supervisor: Jae Il Shin

Thesis Committee Member#1: Ji Hong Kim

Thesis Committee Member#2: Tae-Sun Ha

Thesis Committee Member#3: Hyeon Joo Jeong

Thesis Committee Member#4: Dong Soo Kim

The Graduate School

Yonsei University

June 2013

ACKNOWLEDGEMENTS

“But by the grace of God I am what I am, and his grace to me was not without effect. No, I worked harder than all of them--yet not I, but the grace of God that was with me.” (1 Corinthians 15:10)

As confessed by the Apostle Paul, I also cannot help but confess that I am what I am completely by the grace of God. Above all, I want God the Father in heaven, who loves me and grants me life, strength, health, and wisdom, to be glorified by this accomplishment.

I am extremely grateful to my advisor, Prof. Jae Il Shin, whose stimulating suggestions and encouragement helped me throughout my graduate school years. Special thanks are extended to the PhD Thesis committee for their invaluable advice and feedback during the experiment and revision process. I would also like to extend my gratitude to Prof.

Tae-Sun Ha and his experimental technicians, whose help allowed me to successfully finish my experiment.

Grateful appreciation is also offered to my loving parents, my parents-in-law, my two sisters, and my brother-in-law for their persistent and precious support and encouragement. Finally, I would like to express and extend my warmest, sincerest, and endless love and thanks, through this paper, to my wife, Eun Myung Kang, who gave birth to my son, Kye Myeong Park, who has been there through the most difficult times, who has devoted more than 18 years to me, with whom I hope to spend the rest of my life, and together with whom I hope to go to heaven forever.

June 2013

Se Jin Park, M.D.

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

Interleukin-13 may increase podocyte permeability via modulation of zonula occludens-1

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Purpose: The aim of this study was to investigate whether pathologic changes in zonula occludens-1 (ZO-1) are induced by interleukin-13 (IL-13) in the experimental minimal-change nephrotic syndrome (MCNS) model and to determine whether montelukast, a leukotriene receptor antagonist, has an effect on ZO-1 production in cultured human podocytes.

Materials and methods: Human podocytes cultured on bovine serum albumin-coated plates were treated with different doses of IL-13 and

montelukast and examined for permeability using monolayer semi-permeable membranes, for distribution using confocal microscopy, and for ZO-1 protein levels using Western blotting.

Results: At higher doses, IL-13 gradually increased the permeability of monolayered podocytes. The redistribution and rearrangement of ZO-1 by IL-13 was observed in immunofluorescence studies. ZO-1 was internalized and shown to accumulate in the cytoplasm of human podocytes in an IL-13 dose-dependent manner. High doses (50 and 100 ng/mL) of IL-13 decreased the levels of ZO-1 protein at 12 and 24 hr (both $P < 0.01$; n=3), which were significantly reversed by a high dose (0.5 μM) montelukast treatment ($P < 0.01$, n=3).

Conclusions: Our results suggest that IL-13 may increase podocyte permeability through the modulation of ZO-1, and such alterations in the content and localization of ZO-1 may be relevant to the pathogenesis of proteinuria in the IL-13-induced MCNS model. In addition, the leukotriene receptor antagonist montelukast may be a potential therapeutic option for the treatment of IL-13-induced MCNS.

Key words: interleukin-13, zonula occludens-1, podocytes, leukotriene receptor antagonists

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I. INTRODUCTION

Although minimal-change nephrotic syndrome (MCNS) is the most common cause of nephrotic syndrome during childhood, the various proteins involved in the morphological and functional changes of cytoskeletal proteins in MCNS are still elusive.[1] The hallmark of MCNS pathology is the foot process (FP) effacement of podocytes. Physiologically, FPs remain wide open to serve as a passage for glomerular filtrate and are held together by tenuous slit diaphragms

(SDs) that connect the filtration slits.[2] Glomerular SDs, a major selective barrier for proteins, reside in the cell-to-cell contact sites between neighboring podocytes, which is thought to be a modified adherens junction that consists of an increasing number of proteins, including nephrin, CD2-associated protein (CD2AP), Neph-1, -2, and -3, podocin, and zonula occludens-1 (ZO-1).[3]

Recent studies have identified that increased interleukin-13 (IL-13) expression can lead to podocyte injury and induce a minimal-change-like nephropathy.[4-6] Notably, Lai et al. reported that overexpression of IL-13 caused downregulation of nephrin, podocin, and dystroglycan that are important molecules in maintaining SD integrity, and a concurrent upregulation of B7-1 as well as MCNS in a rat experiment model.[7] Although receptors for IL-13 such as IL-13Rα1 and IL-13Rα2 have also been demonstrated in cultured podocytes,[8] the precise role of IL-13 is still not clear in the pathogenesis of MCNS, and there has been few report on the effect of IL-13 on cultured human podocytes *in vitro*.

While ZO-1 was originally identified as a tight junction component, this molecule was also localized at adherens junctions (AJs) in podocytes.[9-12] ZO-1 is a cytosolic scaffold that connects the cadherin-catenin complex and actin-based cytoskeletons.[9] It is thought to

play a key role in AJs through its interaction with various adherens junction proteins and the formation of multimolecular complexes.[10] Therefore, we hypothesized that IL-13 may play an important role in the development of proteinuria in MCNS by exerting a direct effect on ZO-1 in human podocytes. The aim of our study was to investigate the role of IL-13 and a leukotriene receptor antagonist (LTRA), montelukast, that inhibits the effect of leukotriene influenced by IL-13, on ZO-1 proteins in MCNS.

II. MATERIALS AND METHODS

1. Cell culture of human podocytes

Human conditionally immortalized podocytes (AB8/23), primarily cloned from human glomerular cultures, were characterized and generously provided by Dr. Moin A. Saleem (University of Bristol, Bristol, UK). Human podocytes were maintained in RPMI 1640 (WelGENE, Daegu, Korea) supplemented with 10% heat-inactivated fetal bovine serum (FBS), Insulin-Transferrin-Selenium-Pyruvate Supplement (ITSP; WelGENE), and antibiotics. Fresh media was supplied once every two days.

To stimulate human podocyte proliferation, cells were cultivated at 33°C (permissive conditions) in a culture medium supplemented human recombinant ITSP to induce expression of temperature-sensitive large T antigens. To induce differentiation, podocytes were maintained at 37°C (non-permissive conditions) for at least two weeks. For subculture, 0.05% trypsin was used to detach cells from the culture dishes.[13]

2. IL-13 and montelukast treatment conditions

To imitate MCNS-like conditions, cells were incubated with various

concentrations of IL-13 (Sigma-Aldrich Inc., St. Louis, MO, USA) during the indicated time periods (6, 12, and 24 hr). IL-13 was administered with 3, 10, 30, and 100 μg doses, respectively, into 0.5% RPMI with montelukast at 37°C.

3. Scanning Electron Microscopy

A Hitachi S-570 scanning electron microscopy (SEM; Hitachi, Tokyo, Japan) was used to view samples for SEM. The collagen-coated and differentiated cells were fixed in 5% glutaraldehyde in distilled water, incubated at 4°C for 1 hr, rinsed three times in phosphate buffered saline (PBS), and then dehydrated in a graded series of ethanol solutions (60%, 70%, 80%, 95%, and 100% ethanol) for 7 min in each solution. The same process was performed once more with the exception of fixation in 1% osmium tetroxide in distilled water. The cells were dried before coating with gold and observed by a SEM.

4. Monolayer permeability assay

Podocytes were seeded and grown to confluence in a monolayer pattern on the surface of 0.45 μm cellulose semi-permeable membranes (Millicell-HA, Millipore Corp., Bedford, MA, USA) in 10% RPMI.

Hydrostatic pressure was applied continuously from lower to apical to basolateral aspect. Treated cells were incubated with various concentrations of IL-13 during the indicated 24-hr period in 0.5% RPMI. Then, 1 mg/mL FITC-tagged anionic dextran (Invitrogen, Eugene, OR, USA) was added to the apical media, and the filtered amounts of dextran at each incubation time (2, 4, 6, 8, 16, and 24 hr) were measured by spectrophotometry at 492 nm.

5. Immunofluorescence staining

Human podocytes that were grown on type I collagen-coated glass cover slips were incubated at 37°C for 2 hr and fixed in 4% paraformaldehyde for 20 min. The cells were then permeabilized in 0.1% tritonX-100 for 10 min, blocked with 10% FBS for 30 min, washed 3 times for 5 min in PBS, and labeled with monoclonal rabbit anti-ZO-1 antibody (Invitrogen) and monoclonal goat anti-CD2AP antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Primary antibody-bound specimens were incubated with 1:1000 (v/v) Alexa 594 for red conjugates and Alexa 488 for green (Invitrogen), respective of secondary anti-rabbit IgG, at room temperature for 40 min and at 37°C for 20 min without CO2. Nuclei were stained with 4'-6-diamidino-2-phenylindole (DAPI) (1:1000) for 20 min

in PBS. Coverslips were mounted in aqueous mountant and viewed with a fluorescence microscope (BX51; Olympus, Tokyo, Japan).

6. Western blotting

Confluent cell layers were incubated with additives for various time durations and proteins were extracted using a protein extraction solution (PRO-PREP, Intron, Seongnam, Kyeonggi, Korea) containing phenylmethylsulfonyl fluoride, ethylenediamine tetraacetic acid, pepstatin A, leupeptin, and aprotinin; protein concentrations were then determined as previously described.[14] To perform Western blotting for ZO-1, 30 μg of boiled extracts were resolved on 10% SDS-PAGE gels and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories, Hercules, CA, USA).

The membranes were then air-dried and blocked in 5% fat-free milk before incubation with monoclonal rabbit anti-ZO-1 (Invitrogen). Anti-β-tubulin antibody (Santa Cruz Biotechnology) was used as a loading control. After incubation with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology), protein bands were detected using the ECL (WEST-ZOL® plus; Intron) chemiluminescence detection system (Amersham Biotech Ltd., Little Chalfont, Bucks, UK).

Density values were expressed as percent of the control. Data on densitometric analysis of the ZO-1/β-tubulin ratio are expressed as the mean ± standard deviation (SD).

7. Statistical analysis

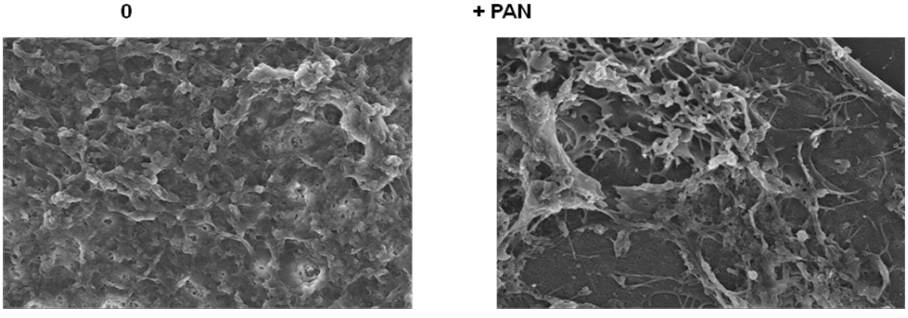
Results were described as the mean ± SD, as appropriate under different conditions. Statistical significance was evaluated by the non-parametric Kruskal-Wallis analysis or Student *t*-test. *P* values < 0.05 were considered significant.

III. RESULTS

1. Ultrastructural changes in human podocytes by IL-13

Under SEM, there seemed to be podocytes with dense and tufted structures in the differentiated condition, but the cells displayed fewer distinct processes or projections in puromycin aminonucleoside (PAN)-induced nephropathy (Fig. 1A). As in PAN-induced nephropathy, SEM ultrastructural analyses also revealed a progressive decrease in distinct processes or projections of the human podocytes after treatment with IL-13, particularly documented at 100 ng/mL of IL-13 (Fig. 1B). Effaced foot processes are from human podocytes treated with PAN and a high dose of IL-13 to induce an experimental nephrotic syndrome.

(A)



(B)

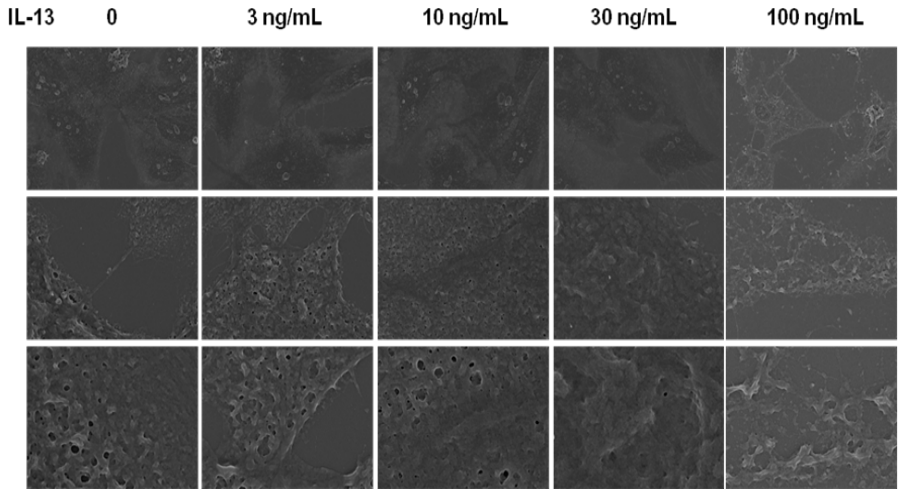


Figure 1. Morphological analysis by scanning electron microscopy (SEM). Under SEM, there were podocytes with dense and tufted structures in the differentiated status (left), but the cells displayed fewer distinct processes or projections in puromycin aminonucleoside (PAN)-induced nephropathy (right) (A). SEM ultrastructural analyses also revealed a progressive decrease in distinct processes or projections of the human podocytes after treatment with IL-13, particularly documented at 100 ng/mL of IL-13 (B). Magnification: 20,000 × in A; 3,000 × in the first row of B, 20,000 × in the second row of B, and 60,000 × in the last row of B, respectively.

2. Changes in cell permeability due to IL-13

High concentrations (100 ng/mL) of IL-13 increased podocyte permeability *in vitro* to peak levels greater than 250% compared to the control (without IL-13) at 6 hr, and then levels decreased to be similar to low concentrations of IL-13 by 8 hr (Fig. 2). IL-13 the doses ≥3 0 ng/mL significantly increased the permeability of monolayered podocytes for 24 hr compared to the untreated control. These findings imply that IL-13 mainly increases podocyte permeability at early stages (2-6 hr); after this time, the permeability continues to increase due to senescence of the podocytes.

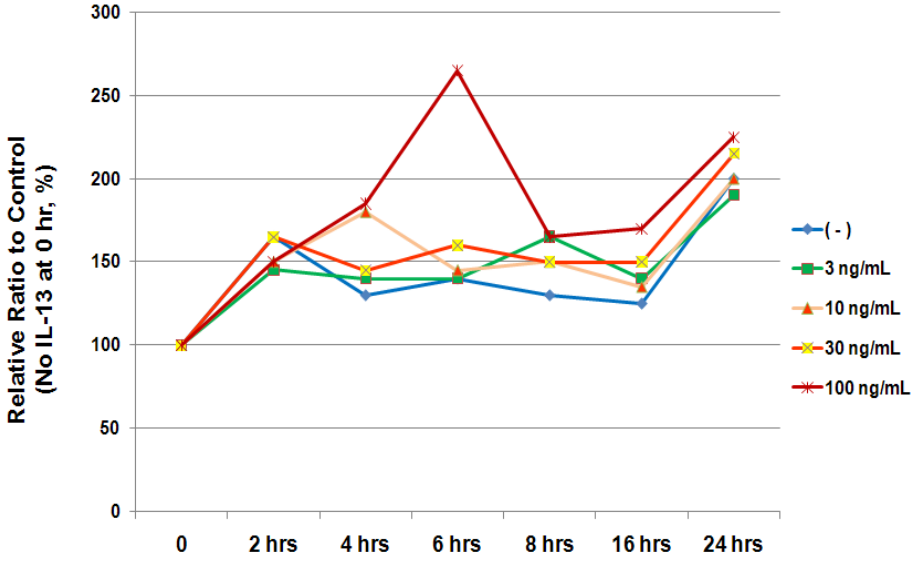


Figure 2. Effects of IL-13 on increased monolayer podocyte

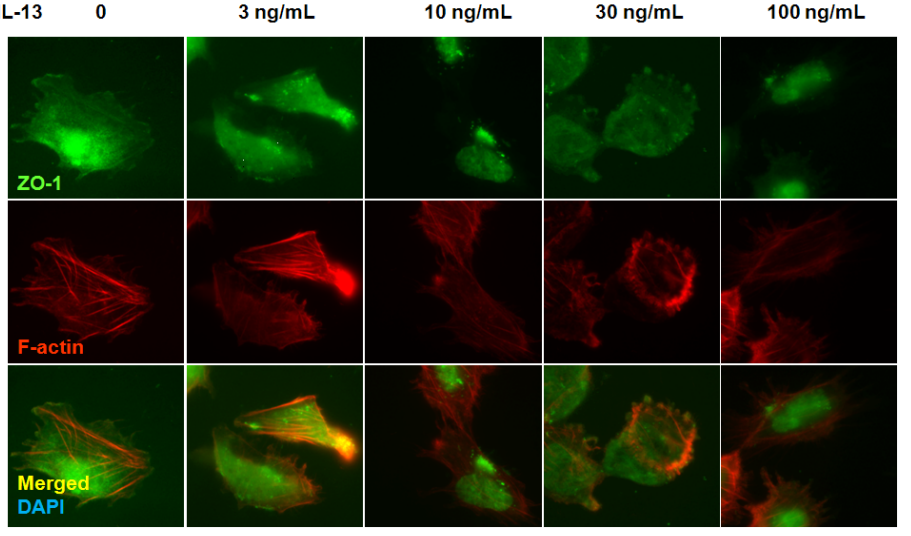
permeability. Podocyte permeability reached its peak at 6 hr at 100 ng/mL IL-13 and significantly increased for 24 hr in the groups treated with ≥30 ng/mL IL-13.

3. ZO-1 distribution on confocal microscopy

Podocytes were double-stained for ZO-1 and F-actin, and the cell nuclei were stained with DAPI. ZO-1 in human podocytes was highly expressed within the podocyte in the cytoplasmic aspect of the FP membrane, adjacent to the insertion of the SD, and colocalized with β-catenin (Fig. 3A). Staining for ZO-1 was most intense in the cytoplasmic surface of the podocyte FP. ZO-1 and F-actin did not overlap and were located at the different sites in the confocal images. From low to high IL-13 concentrations, ZO-1 staining became blurry, which indicates a relocalization of ZO-1 away from the peripheral cell membrane. In the human podocytes under IL-13 conditions, ZO-1 was internalized into the cytoplasm from the peripheral cell membrane as IL-13 concentrations increased (Fig. 3A). These distributional changes were also observed in F-actin, particularly at the higher IL-13 concentrations (Fig. 3A). Moreover, in

high-resolution microscopy, ZO-1 was distributed to the cell contact areas under physiologic conditions without IL-13 but was redistributed and accumulated into the cytoplasm around the nucleus during a 6 hr-incubation period as IL-13 increased from 0 ng/mL to 30 and 100 ng/mL (Fig. 3B). These results suggest that IL-13 may have a substantial impact on the redistribution and rearrangement of ZO-1 molecules and disrupts the cytoskeletal connections between F-actin and α-catenin-β-catenin complex in a concentration-dependent manner (Fig. 3B). The internalized ZO-1 proteins were restored to the periphery by treatment with a high dose of 0.5 μM montelukast (Fig. 3B).

(A)



(B)

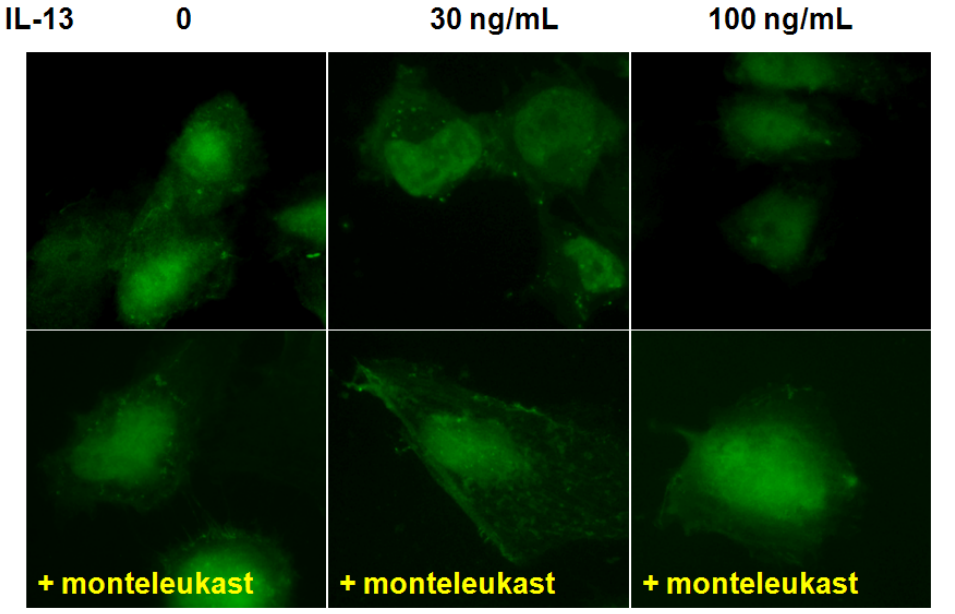


Figure 3. Distributional changes in ZO-1 by IL-13 in human podocytes. ZO-1 was distributed at the peripheral cell membrane and colocalized with β-catenin and actin filament at cell-to-cell contact junctions. High concentrations of IL-13 suppressed and disrupted the immunostaining and linearity of ZO-1 proteins, and accumulated ZO-1 proteins into the cytoplasm around nucleus (A), which improved by treatment with 0.5 μM montelukast (B). Magnification: 1,000 x ; Scale bar = 20 μm.

4. ZO-1 protein assayed by Western blotting

A major ZO-1 protein band was found at 205 kDa and ZO-1 levels were compared with β-tubulin. In human podocytes, density values for ZO-1 protein tended to decrease with IL-13 treatment in a dose-dependent manner at 12 and 24 hr. A high dose (100 ng/mL) of IL-13 significantly decreased the amount of ZO-1 protein by 37.5% at 12 hr and by 39% at 24 hr (both $P < 0.01$; n=3) (Fig. 4). Fifty ng/mL IL-13 significantly decreased the amount of ZO-1 protein by 31.0% at 12 hr and by 34.9% at 24 hr (both $P < 0.01$; n=3) (Fig. 4). Ten ng/mL of IL-13 also decreased ZO-1 protein levels to a greater degree by 35.1% at 24 hr ($P < 0.05$) than by that at 12 hr. The reduced ZO-1 protein levels by 30 ng/mL of IL-13 was restored by a high dose of 0.5 μM montelukast ($P < 0.01$; n=3), but was not affected by 0.2 μM of montelukast (Fig. 5). These results suggest that IL-13 may induce a minimal-change-like nephropathy through a reduction in ZO-1 molecules, which can be reversed by high dose of the LTRA montelukast.

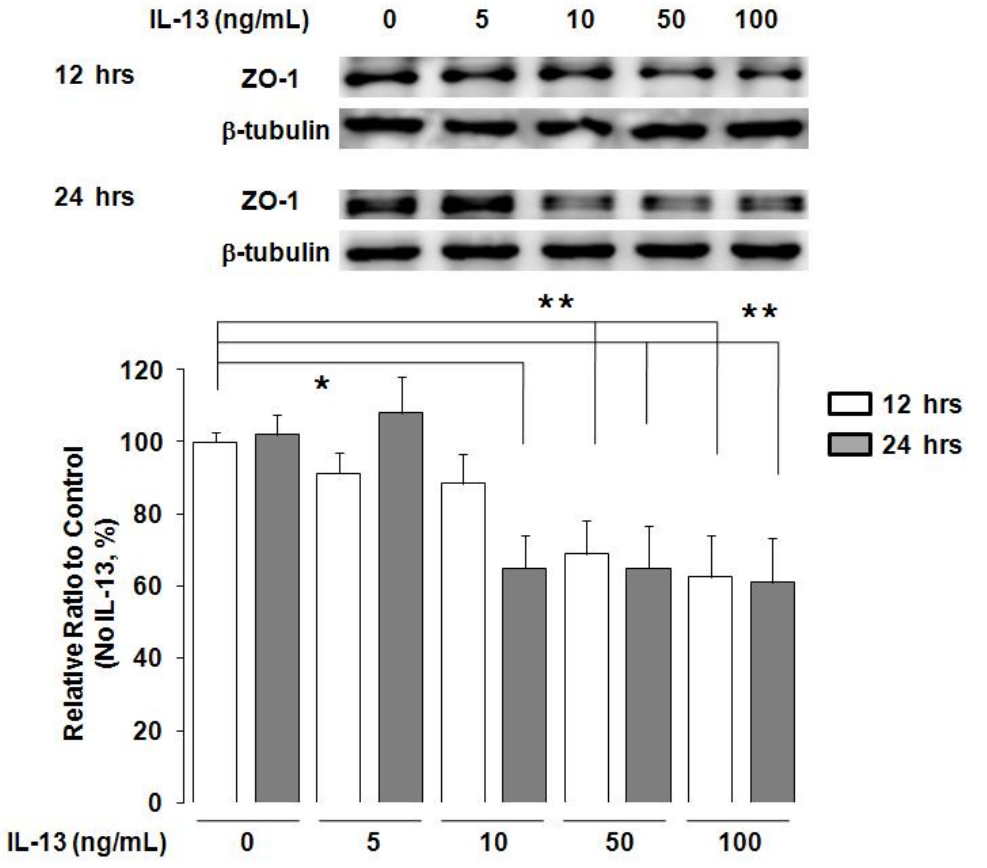


Fig. 4. Effects of IL-13 on ZO-1 protein levels in cultured human podocytes as assayed by Western blotting. ZO-1 levels were significantly decreased at IL-13 concentrations of more than ≥ 50 ng/mL at 12 and 24 hr incubations, compared with the control. Data on the densitometric analysis of the ZO-1/β-tubulin ratio are expressed as the mean ± SD. Control (100%): the value of (-). *$P < 0.05$, **$P < 0.01$.

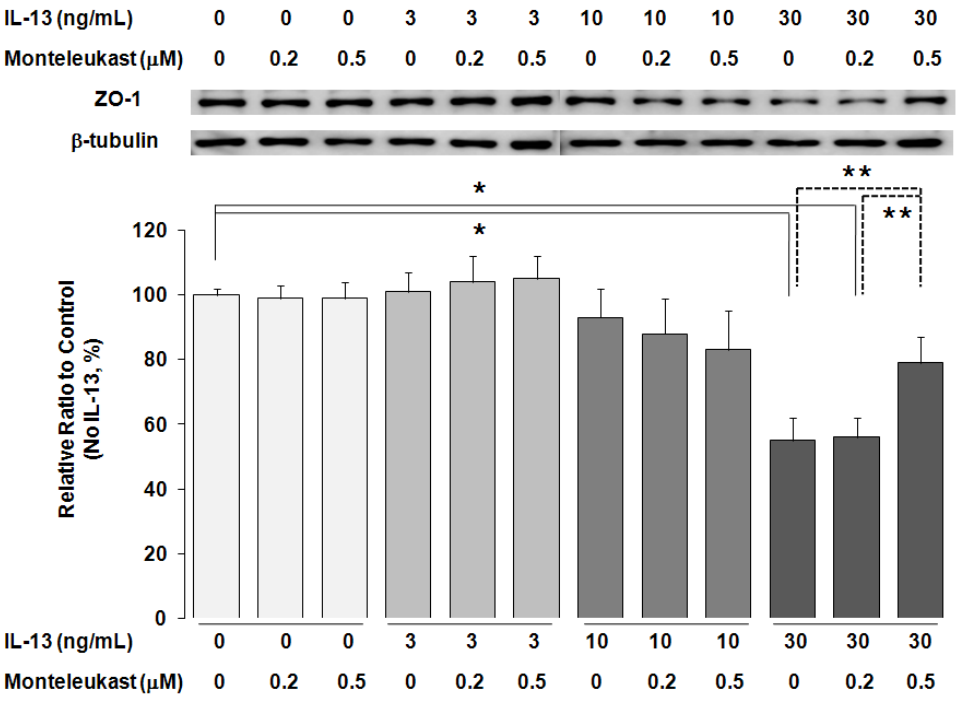


Fig. 5. Effects of montelukast on ZO-1 protein levels in cultured human podocytes assayed by Western blotting. Montelukast (0.5 μM) significantly increased ZO-1 levels in human podocytes treated with IL-13. Data on the densitometric analysis of the ZO-1/β-tubulin ratio are expressed as the mean ± SD. Control (100%): the value of (-). *$P < 0.05$, **$P < 0.01$.

IV. DISCUSSION

The main goal of this study was to determine whether pathological changes in ZO-1 protein levels in MCNS could be induced by IL-13. We demonstrated an increased permeability, redistribution, and reduction in ZO-1 proteins from human podocytes treated with IL-13. The exposure of ZO-1 molecules to IL-13 caused ZO-1 to move and accumulate internally toward the cytoplasmic actin filaments, suggesting that the observed hyperpermeability, redistribution, and reduction in ZO-1 proteins could be involved in the pathogenesis of MCNS. These results are similar to our previous studies that ZO-1 proteins in podocytes were also affected by diabetic conditions, causing hyperpermeability at early stages.[15, 16]

Recent studies have shown strong evidence that proteinuria in MCNS is associated with cytokines and T cell disorders that result in glomerular podocyte dysfunction,[4, 5, 17] as well as B7-1 (CD80) that is expressed on the surface of B cells. An increase in IL-13 production by CD3+, CD4+, and CD8+ T cells was shown to mediate steroid-sensitive nephrotic syndrome in relapse.[4, 5] Of note, Lai et al. demonstrated that IL-13-transfected rats developed a minimal-change-like glomerulopathy,

as characterized by significant worsening of albuminuria over time, generalized swelling, low serum albumin, and hypercholesterolemia.[7] In IL-13-transfected rats, light microscopy showed the absence of significant glomerular changes; however, electron microscopy revealed up to 80% effacement of podocyte FPs, which progressed the nephrotic syndrome.[7] Although the nephrotic-range proteinuria was selective in IL-13-transfected rats, no relationship between serum IL-13 and urinary albumin excretion or serum albumin levels was observed.[7] Thus, our study attempted to define the pathogenic relationship between IL-13 and ZO-1 protein in the development of podocyte FP effacement, with nephrotic-range proteinuria, especially in human podocytes.

The proteinuric conditions in MCNS are usually associated with ultrastructural changes in the podocytes with fusion, widening, retraction, and gradual simplification of the highly specialized interdigitating FP, which are also accompanied by alterations in the permselectivity of the SD glomerular filtration barrier and the linking of adaptor proteins, including nephrin, podocin, CD2AP, catenins, and ZO-1.[17-19] Maintenance of podocyte FP structure is pivotal for accurate functioning of the glomerular filtration barrier. The FP effacement results from the detachment of podocytes from the glomerular basement membrane

(GBM) and FP retraction leads to disintegrity of the cytoskeletal structure and linking adaptor proteins, abnormal movement of FP over the GBM, and reconstruction of the SD.[3, 18] Permselectivity of the glomerular filtration barrier, which is composed of a charge-selective barrier and a size-selective barrier, also plays a key role to restrict passage of plasma proteins across the GBM and SD.[20, 21]

Zonula occludens (ZO), which is a member of the membrane-associated guanylate kinase (MAGUK) homologue family of proteins that are characterized by their PSD-95/discs-large/Zonula occludens-1 (PDZ) domain, are critical regulators of tight and adherens junction assembly.[22] ZO connects some of the SD proteins through its PDZ domain to the actin cytoskeleton. Although ZO-1 was originally identified as a tight junction component, ZO-1 migrates from its apical location down to the level of the slit membrane at the capillary loop stage of renal development, where it is observed in a punctuate pattern along the filtration slits.[23] There are three ZO proteins, ZO-1, -2, and -3, which are multi-domain polypeptides.[10] Of these three molecules, ZO-1 has been proposed to be a scaffolding protein between transmembrane and cytoplasmic proteins, and possibly forms a link between actin and the cadherin-catenin complex.[22] ZO-1 may also participate in signaling

events through tyrosine phosphorylation,[24] and binds directly to α-catenin and actin, respectively, located on the FPs at the insertions of the SD. [23, 25] ZO-1 forms a gasket that seals off intercellular spaces and restricts the movement of proteins, water and solutes along the paracellular pathway at tight junctions, maintaining the polarized distribution of membrane proteins.[22] However, the functions of ZO-1 between podocyte FPs are not to serve as a seal but to firmly attach FPs to one another and to stabilize the FP layer against a high filtration pressure at the SD, a modified adherens junction rather than a derivative of the tight junction.

Downregulation of the glomerular gene and production of SD proteins such as nephrin, podocin, and dystroglycan has been reported in both experimental models of nephropathy such as puromycin nephropathy or adriamycin nephropathy, and human kidney biopsies.[26-28] In these studies, immunofluorescence staining showed reduced protein production and staining intensity for nephrin, podocin, and dystroglycan, with a shift from a linear to a discontinuous and granular pattern, that returned to normal after steroid treatment. Moreover, a few reports showed that changes in the properties or production of ZO-1 might accompany renal diseases associated with proteinuria.[29, 30] However, very little is known about how binding of proteins to ZO-1 is regulated or how cell-signaling

pathways control adherens assembly and filtration barrier function, although many different pathways have been implicated.[31] Thus, this study explored the production of ZO-1 and the effect of IL-13 and leukotriene receptor antagonist on ZO-1 production in cultured human podocytes. While Kurihara et al. did not show a quantitative change in ZO-1 protein production in a PAN-treated rat model,[30] we found the redistribution and reduction in ZO-1 protein in IL-13-induced MCNS. In addition, IL-13 significantly decreased ZO-1 protein levels in human podocytes of IL-13-induced MCNS, whereas ZO-1 protein production was significantly increased in the rat models of puromycin aminonucleoside nephrosis.[32]

The present study has some limitations: 1) we were unable to demonstrate all of the signaling pathways of IL-13 via IL-13 receptor (a heterodimer of IL-4Rα and IL-13Rα1) to ZO-1 proteins in podocytes, presumably cascading through tyrosine phosphorylation of ZO-1; 2) although IL-13 is known to influence leukotriene levels and IL-13-1112C/T polymorphism and the haplotype of IL-13 polymorphisms are known to be significantly associated with LTRA drug responsiveness,[33] the mechanism of montelukasts in IL-13-induced MCNS is not fully understood yet; and 3) the efficacy of montelukast as

a useful, optional add-on treatment in MCNS should be proven in *in vivo* experiments to reduce massive proteinuria and potential side effects from corticosteroids. Despite these limitations, the current study demonstrated the potential of LTRA on reduced ZO-1 proteins in an IL-13-induced MCNS model of human podocytes, whereas treatment with angiotensin-converting enzyme inhibitors ameliorated proteinuria and restored the normal localization of ZO-1 at the SD in spontaneously proteinuric Munich-Wistar-Froemter rats.[34] Additional, further studies are necessary to elucidate the exact mechanisms, efficacy and proper dose of LTRA in the *in vivo* treatment of IL-13-induced MCNS.

In conclusion, our study provides one of the bases for understanding the ZO-1 molecule in human podocytes of IL-13-induced MCNS. High concentrations of IL-13 increased podocyte permeability, suggesting the disintegrity and disruption of glomerular filtration barrier in SD, and FP effacement. ZO-1 proteins were redistributed, relocated, rearranged, and reduced in IL-13-induced MCNS, which was significantly restored after treatment with a leukotriene receptor antagonist. Therefore, our findings further strengthen the hypothesis that IL-13 may increase podocyte permeability through modulation of ZO-1 proteins, resulting in nephrotic-range proteinuria, namely MCNS, and also provide an

explanation for the plausible connection among Th2 cytokines, MCNS, and atopy.

V. CONCLUSION

Our results suggest that IL-13 may increase podocyte permeability through the modulation of ZO-1, and such alterations in the content and localization of ZO-1 may be relevant to the pathogenesis of proteinuria in the IL-13-induced MCNS model. In addition, the LTRA montelukast may be a potential therapeutic option for the treatment of IL-13-induced MCNS.

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ABSTRACT (IN KOREAN)

Zonula occludens−1의 변화를 통한 interleukin-13의 족세포 투과도 증가

<지도교수 신 재 일>

연세대학교 대학원 의학과

박 세 진

목적: 본 연구는 인터루킨−13에 의해 유도된 실험적 미세변화 신증후군 모델에서 zonula occludens−1 (ZO−1) 단백질에 어떤 병리적 변화가 유발되는지와 루코트리엔 수용체 길항제인 몬테루카스가 배양된 사람 족세포에서 ZO−1의 재생성에 영향을 미치는지 알아보고자 시행되었다.

방법: 우(牛)혈청 알부민이 코팅된 플레이트에서 배양된 사람 족세포를 인터루킨−13과 몬테루카스의 각각 다른 용량으로 처

리한 후, 단층 반투과막을 이용하여 ZO-1의 투과도를, 공초점 현미경을 사용하여 ZO-1의 분포를, 웨스턴 블로팅법을 사용하여 ZO-1 단백질의 수치를 각각 조사하였다.

결과: 인터루킨-13은 농도가 높아질수록 단층 족세포의 투과도를 점점 증가시켰다. 인터루킨-13에 의한 ZO-1의 재분포와 재배열은 면역형광염색에서 관찰되었다. ZO-1은 인터루킨-13에 용량-의존방식으로 사람 족세포의 세포질내로 내재화 하였으며 세포질에 축적되는 경향을 보였다. 고농도(50과 100 ng/mL)의 인터루킨-13은 12시간과 24시간 후 ZO-1 단백질의 수치를 유의하게 감소시켰으며(양쪽 모두 $P < 0.01$; n=3), 감소된 수치는 또한 고농도(0.5 μM)의 몬테루카스에 의해 유의하게 회복되었다($P < 0.01$, n=3).

결론: 본 연구 결과는 인터루킨-13이 ZO-1 단백질의 변화를 통하여 족세포의 투과성을 증가시킬 수 있으며, 이러한 ZO-1의 양적, 위치적 변화가 인터루킨-13에 의해 유도된 미세변화 신증후군 모델에서 단백뇨의 병인과 연관이 있을 수 있다고 제시한다. 덧붙여, 루코트리엔 수용체 길항제인 몬테루카스트가 인터루킨-13에 의해 유도된 미세변화 신증후군의 치료에 있어

하나의 잠재적인 보조대안이 될 수 있다는 것을 보여준다.

핵심되는 말: 인터루킨-13, zonula occludens-1, 족세포, 류코트리엔 수용체 길항제