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Effects of Ketotifen on an Experimental Model of IgA Nephropathy

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= **Abstract** =

Purpose : The intestinal mucosal defect has been known as one of the pathogenicmechanisms of IgA nephropathy. Oral antigens usually induce the activation of Th2 cells and mast cells. These cells secrete cytokines IL-4, IL-5 and TGF-β, which increase IgA production. Although ketotifen (benzocycloheptathiophene) is an H1 antagonist and a mast cell membrane stabilizer, it could protect the gastrointestinal membrane through inhibiting the production of IL-4, IL-5, PGE2, and LTB4, and decreasing the activity of nitric oxide synthease. Therefore, we have investigated if ketotifen may protect the development of IgA nephropathy with an oral antigen.

Methods : ICR mice were used as an animal model orally with Poliovax only [ketotifen (-)], the other group was given oral ketotifen [ketotifen (+)] in addition to Poliovax.

Results : Mesangial IgA deposition developed in 11 out of the 18 mice in the ketotifen (-) group, while in three out of the nine mice in ketotifen (+) group. The mesangial change developed in 16 out of the 18 mice in the ketotifen (-) group, while in five out of the nine mice in the ketotifen (+) group. Serum IL-4 and IL-5 levels were not significantly lower in the latter group than in the former.

Conclusion : According to the statistical results from the above, ketotifen therapy would be beneficial to reducing mesangial changes in IgA nephropathy. **(J Korean Soc Pediatr Nephrol 2009;13:153-160)**

Key Words : Experimental IgA Nephropathy, IL-4, IL-5, Benzocycloheptathiophene (Ketotifen)

Introduction

After intraoral antigen presentation, the cytokines were secreted from the mast cells and Th2 cells, producing TGF-β which promote production of IgA from the Peyer's patch [1,2]. That is, increasing the IL4, IL-5 and IL-6 which are the cytokines of the Th2 cells from the T cells and mast cells, is effective to the intestinal IgA production. Excessive IgA production was known as the one of the causative mechanism of IgA nephropathy. Benzocycloheptathiophene (Ketotifen), which has been used as an anti-allergic drugs for a long time, is a H1 receptor antagonist and mast cell stabilizer. It was found to protect

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the gastric and intestinal mucosa through inhibition of production of IL-4 and IL-5, decreasing PGE2 and LTB4, and increasing the activity of NOS. Thus, ketotifen has been used as the drug for the gastrointestinal diseases, such as, NSAID induced gastritis and irritable bowel disease since 1990′ [3-5].

We formulated the hypothesis that experimental IgA nephropathy with oral antigen and simultaneous ketotifen will show decreases in intestinal IgA production and IgA nephropathy. Therefore, we have developed the experimental IgA nephropathy with oral polio vaccine and simultaneous ketotifen.

Materials and Methods

1. Materials

ICR mice (5 week-old, weight 20-30 g) were used. We classified the experimental groups as ;

Control group C: 10 mice fed with a normal diet.

Vaccine group V: 20 mice given OPV by tube

Ketotifen group V+K: 10 mice given OPV by tube and added water diluted with ketotifen for whole phase (Fig. 1)

2. Methods

1) Administration of antigen

OPV (Poliovax, Green Cross, Seoul), Polio virus type I $10^{6.0}$ $TCID_{50}$, polio virus type II $10^{5.0-5.3}$ $TCID_{50}$, polio virus type III $10^{5.3-5.7}$ $TCID_{50}$, 0,2 mL were introduced via intragastric tube 3 times in the experimental groups, on the 1st day, the 30th day and the 70^{th} day.

2) Administration of benzocycloheptathiophene

Water was supplied daily in amounts of 15 mL/100 g of mouse body weight till the last day of the experiment. Benzocycloheptathiophene was diluted to the concentration of 1 mg/100 mL water (150 μg/100 g B.Wt/day).

3) Organ harvest and immunochemistry

All mice were sacrificed on the experimental 100^{th} day. Kidney, small intestine and skin were immunohistochemically stained with H&E stain, PAS, silver stain, FITC-conjugated antimouse IgA and IgE (Sigma, St, Louis, USA). Serum IL-4 and IL-5 were measured with anti-mouse IL-4 and IL-5 neutralizing antibody via ELISA.

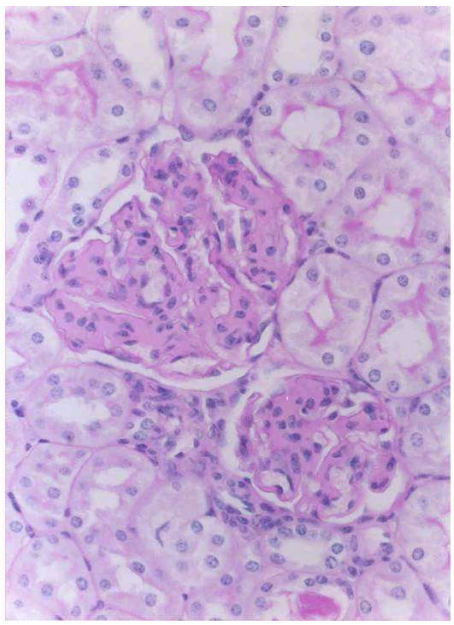

Fig. 1. Two glomeruli showing moderate mesangial widening and mild proliferation of mesangial cells (PAS stain, ×400).

4) Data analysis and statistical analysis

The results were expressed as mean plus or minus standard error. Differences between variables were analyzed by Student t-test and the analysis of variance. A P value of less than 0.05 was considered statistically significant.

Results

1. Pathological finding

1) Gross finding

During the experiments, one mouse in group C died, one mouse in group V died of intratracheal administration of OPV. There was one mouse with a kidney abscess in group V and group V+K, separately. They were all excluded from the data analysis.

For the final data analysis, there were 9 mice in group C, 18 mice in group V, and 9 mice in group V+K.

2) Microscopic finding

(1) Rate of mesangial matrix and cell proliferation

The pathologic findings were analyzed with injury scoring system (normal=1, focal change=2, diffuse change=3). There were no mesangial abnormalities in group C (score: 1.000±0.000). Sixteen out of 18 (88.9%) in group V (score: 2.1176 ±0.169) were found to have mesangial abnormality. Five out of 9 (55.6%) in group V+K (score: 1.8000±0.1333) were also found to have the mesangial abnormality. The degree of changes in group V+K showed weaker than group V ($P<$

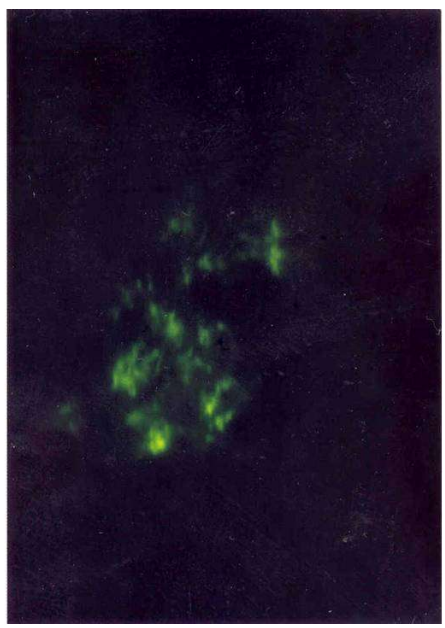

Fig. 2. Mesangial IgA deposition (Immunofluorescent stain, ×200).

Table 1. Comparison of Mesangial and Tubular Injury, IgA Deposition, Intestinal Injuryscore Among groups (mean±S.E)

Score	Mesangial	Tubular	IgA deposition	Intestinal
Group	Injury Score	Injury Score	Score	Injury Score
C	1.0000±0.000	1.0000±0.000*, †	2.3333±0.333*, †, §	1.0000±0.000
V	2.1176±0.169	1.3529±0.119	2.6210±0.183‖	1.3529±0.119
V+K	1.8000±0.1333¶	2.3333±0.289	2.5000±0.866	1.0000±0.000

Injury score: normal =1, focal change=2, diffuse change=3
*$P<0.05$ vs. V; †$P<0.05$ vs. V; ‡$P<0.05$ vs. V; §$P<0.01$ vs. V+K; ‖$P<0.001$ vs. V+K; ¶$P<0.05$ vs. V
Abbreviations : C, control group; V, vaccination only group; V+K, vaccination+ketotifen group

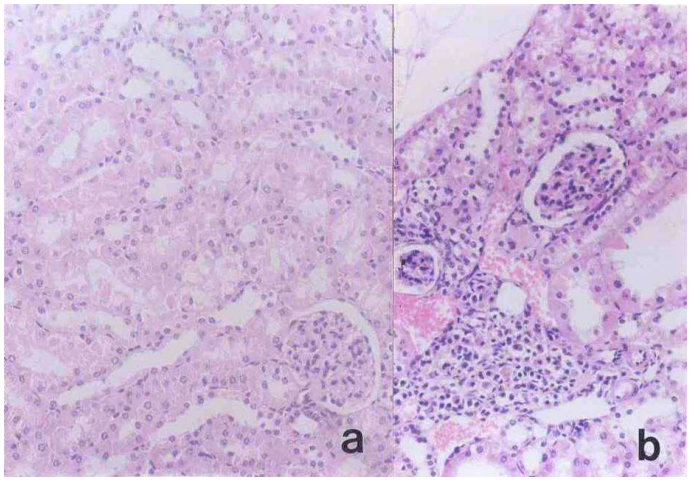

Fig. 3. Tubular degeneration **(a)** and focal interstitial infiltration of mononuclear cells **(b)** (H&E, ×100).

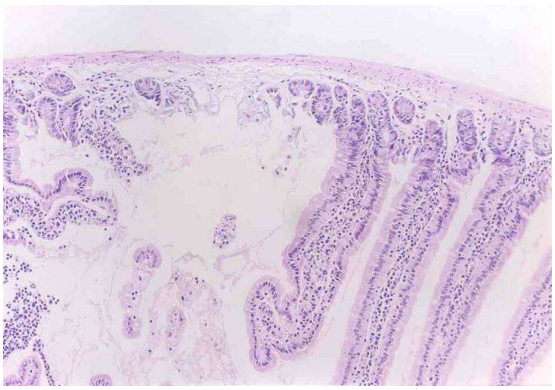

Fig. 4. A focal mucosal necrosis of the jejunum (H & E stain, ×40).

0.05) (Table 1, Fig. 1).

(2) Immunofluoresence microscopic findings

The IgA deposition findings were analyzed with injury scoring system [negative=1, trace=2, (+)=3, (++)=4]. Three of group C had positive findings for IgA (score: 2.333+/-0.333). One had one positive finding, two showed trace finding. In group V, 11 mice (+-++) out of 18 showed IgA deposition (score: 2.6250±0.183). In group V+K, three out of 9 showed IgA deposition(score: 2.5000± 0.866). One had (++), two had (+). (*P* value between V and V+K <0.001) (Table 1, Fig. 2).

(3) Tubulointerstitial change

There were tubular regeneration, atrophy and dilatation. In group C, there were no tubulointerstitial change (1.000±0.000). In group V, 4 out of 18 (1.3529±0.119), in group V+K, 7 out of 8 (2.3333±0.239) showed tubular changes. The greatest change was in group V+K (Table 1, Fig. 3).

(4) Small intestinal injury

The small intestinal changes were only found in group V (8 out of 18 mice). The findings were focal mucosal necrosis, infiltration, focal mucoid sarcoma, and cellular infiltration ($p<0.01$) (Fig. 4).

2. Serum IL-4 and IL-5

1) Serum IL-4

The value of IL-4 markedly increased about 7 times in group V (22.2575±-6.176 pg/mL) rather than group C (3.8450±2.332 pg/mL) ($p<0.01$). Group V+K (18.3100±7.208 pg/mL) showed no specific decreasing effects (Table 2).

Table 2. Comparison of Serum IL-4 and IL-5 Among Groups (mean±SE)

Groups	IL-4	IL-5 (pg/mL)
C	3.8450±.332*	2.0762±0.527†
V	22.2575±.176	4.0067±0.504
V+K	18.3100±7.208†	3.5710±0.869

*P<0.01 vs. V; †P<0.05 vs. V; ‡P<0.05 vs. V+K.
Abbreviations : C, control group; V, vaccination only group; V+K, vaccination+ketotifen group

2) Serum IL-5

In group V, the level of IL-5 (4.0067±0.504 pg/mL) increased to about 2 times over the group C (2.0762±0.527 pg/mL) (P<0.05). Group V+K groups showed increasing value of IL-5 (3.5710±0.869 pg/mL), too (Table 2).

Discussion

The cytokines play a majoy role in the pathogenesis of IgA nephropathy. Increases of plasma cytokines, such as IL-1B, IL-4, IL-5, IL-6, IL-10, IL-12, INF-γ, PDGF-β, NF-kB and IL-1α in the tissue have been reported [6-10]. IL-4 and IL-5 are usually involved to typel hypersensitivity. But in the normal and pathologic renal tissue, there are the autocrine and paracrine pathways of IL-4 in the kidney [11]. Those pathways are in glomerular matrix, epithelial cells and Bowman epithelial cells. Serum IL-4 and IL-5 are involved in the glomerular deposition of IgA in IgA nephropathy via atypical glycosylated IgA [12-14]. The drug benzocycloheptathiophene which is well known as a Th2 cell cytokines inhibitor, is an antagonist of the H1 receptor and mast cell membrane stabilizer, and has been used as an antiallergic drug. But in the '90's, this drug was found to protect gastrointestinal mucosa from injury. Now, it has been found to be effective in protecting against NSAID induced gastric mucosa damage and for the treatment of ulcerative colitis and Crohn's disease [15, 16]. The mechanism was thought to be H1 receptor antagonist and NO production. NO is a modulator of mast cell induced proinflammatory action. Mast cells are found in large numbers in the intestines. Mast cells also contribute to renal damage through local activation of the rennin-angiotensin system in IgA nephropathy [17, 18].

Ketotifen also activates NOS distributed in the renal outer cortex and glomeruli, resulting in a decrease in renal vascular resistance [19, 20].

There was increased intestinal permeability, increased serum IgA against the food antigen, and IgA deposition in the glomeruli in IgA nephropathy [21]. According to the findings, sodium-cromoglycate (SCG), the anti allergic drugs for the food allergy, could protect the experimental animal from IgA nephropathy induction [22-25]. Jin et al [24] said that SCG could prevent the experimental IgA nephropathy. They used the ddy mice for animal model. It was the limitation of their study. Because ddy strain of mouse shows a spontaneous development of IgA nephropathy [26]. In human IgA nephropathy, there could be decreased proteinuria with a high dose (1,200 mg/d) of SCG, but not a significant decrease with low doses (400 mg/d) [22]. SCG is a membrane stabilizer of mast cells and smooth muscle fibers. Ketotifen has a somewhat similar action to mast cells but in addition, acts on the neutrophils and the secretion of cytokines [27].

The experimental IgA nephropathy could usually be done with oral antigen. In general, oral

antigen activated Th2 cell which produce IgA at Peyer's pathch via IL-4, IL-5 and IL-6 [28,29]. But there was no data for the findings regarding intestinal mucosa during the IgA nephropathy experimental modeling.

We made this study with oral antigen and ketotifen. This IgA nephropathy experimental model with the addition of ketotifen, resulted in decreased IgA nephropathy expression. We did not find a decreasing serum IL-4 and IL-5. So we could think ketotifen might be effective in decreasing IgA expression without decreasing serum Th2 cell cytokines, IL-4 and IL-5. As to the place of the site of the IL-4 and IL-5, we suspected that those cytokines did not originate from the intestines. Ketotifen protected the mucosa, with other mechanism because there was marked decreased in intestinal mucosal erosion in the ketotifen group. We found renal tubular atrophy and regeneration more frequently and high levels of IL-4 and IL-5 in the group with ketotifen. From this phenomenon we suspected that ketotifen would protect the GI mucosa, but have toxic effects on the kidney. But in human, cystitis is the only reported urinary tract related adverse effects of ketotifen [30]. Recently, it was found that IL-4 resulted in the protection of glomerular injury by decreasing the glomerular macrophage activity [31]. In our study, decreasing the glomerular damage might result from decreasing the absorption of intestinal antigen. More studies are needed on the adaptation of this result to the IgA nephropathy prevention in human.

요 약

IgA 신증의 실험모델에서 케토티펜의 효과

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목 적 : 장점막 손상은 IgA 신증의 병리기전중의 하나로 알려져 있다. 구강항원은 보통 Th2 세포와 비반세포를 활성화 시킨다. 이러한 세포들은 IL-4, IL-5 TGF-β와 같은 싸이토카인들을 분비하여 IgA 생성을 증가시킨다. 케토티펜(benzpxycloheptathiophene)은 H1항체이자 비반세포의 막안정제로 IL-4, IL-5, PGE2, LTB4 등의 생산을 억제하고, 질산화산소합성제의 활성화를 감소시켜 위장관막을 보호한다. 저자들은 구강항원으로 인한 IgA 신증의 발병을 케토티펜이 예방할 수 있는지 관찰하였다.

방 법 : ICR 생쥐를 이용하여 구강 폴리오백신(백신군)을 투여하면서, 다른 군에서는 케토티펜(케토티펜군)을 백신과 동시에 투여하였다.

결 과 : 메산지움의 IgA 침착은 백신군에서 18마리중 11마리에서 발생하였으나, 케토티펜군에서는 9마리 중 3마리에서 볼 수 있었다. 메산지움의 조직변화는 백신군에서 18마리 중 16마리, 케토티펜 군에서는 9마리 중 5마리에서 볼 수 있었다. 혈청 IL-4, IL-5치는 케토티펜 군에서 백신군과 비교해 다소 낮기는 하지만 의미있는 감소는 하지 않았다.

결 론 : 케토티펜은 IgA 신증의 사구체 변화를 감소시키는데 유효한 것으로 사료된다.

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