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Original Article

The Kidneys of Infant Mice are not Sensitive to the Food Mycotoxin Contaminant Nivalenol

Kaoru Inoue[1*], Miwa Takahashi[1], Yukio Kodama[2], Akiyoshi Nishikawa[3], Yoshiko Sugita-Konishi[4], and Midori Yoshida[1]

[1] Division of Pathology, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158-8501, Japan
[2] Division of Toxicology, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158-8501, Japan
[3] Biological Safety Research Center, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158-8501, Japan
[4] Laboratory of Food and Hygiene, Department of Food and Life Science, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 252-5201, Japan

Abstract: Nivalenol (NIV) is a trichothecene mycotoxin produced by *Fusarium* fungi that frequently contaminates agricultural commodities. Dietary administration of NIV to adult mice affects the renal glomeruli, but data about NIV toxicity in human infants are limited. To evaluate the effects of NIV on infant kidneys, 3-week-old male ICR-derived glomerulonephritis (ICGN) and ICR mice were administered 0, 4, 8 or 16 ppm NIV in diet for 4 weeks, and their renal status was compared with age-matched or adult ICR mice. In ICGN mice, the number of glomeruli showing mesangial expansion and α-smooth muscle actin (SMA)-positive mesangial cells was higher with 16 ppm NIV compared with controls. No other significant differences were observed in ICGN mice. In infant ICR mice, the IgA serum concentrations were significantly elevated without glomerular morphological changes in the 16 ppm NIV group. There was no difference in NIV sensitivity in the kidneys of infant ICGN and ICR mice. These data suggest that the kidneys in infant mice are not sensitive to nivalenol under the present conditions. (DOI: 10.1293/tox.2013-0047; J Toxicol Pathol 2014; 27: 57–66)

Key words : nivalenol, renal glomeruli, ICGN mice, ICR mice, infants

Introduction

Trichothecene mycotoxins produced by *Fusarium* fungi are frequent contaminants in agricultural commodities such as rice, wheat, rye, barley, oats, corn, and other cereals produced in various countries around the world[1–4]. Contamination with these mycotoxins, including *Fusarium* mycotoxins, remains a major concern for human and animal health[5–7].

Nivalenol (NIV), a trichothecene mycotoxin, is produced by strains of the *Fusarium* genus including *Fusarium graminearum* and *Fusarium culmorum*. Toxicities of NIV have been reported that 50 and 100 mg/ml NIV damaged the nuclear DNA of cultured CHO cells in the absence of S9 mix, and oral (20 mg/kg) or intraperitoneal (3.7 mg/kg) administration of NIV to mice resulted in DNA damage in the kidneys, bone marrow, stomach, jejunum, and colon[8]. A low level (0.1–0.5 μM) of NIV also induced DNA damage in differentiated human enterocyte-like Caco-2 cells[9]. On the other hand, other results have shown a negative response to NIV in the Ames test and recombination-repair (rec)-assay[10]. The oral LD_{50} of NIV in mice was determined to be 38.9 mg/kg body weight[11]. Significant leukopenia and growth retardation were observed in a chronic toxicity study of female mice[11]. In a two-year feeding study of female mice, NIV was not tumorigenic, although growth retardation and leucopenia were observed[12]. Based on these toxicological data, the lowest-observed-adverse-effect level (LOAEL) was concluded to be 0.7 mg/kg body weight/day, and the temporary tolerable daily intake (t-TDI) of NIV was set to 0 to 0.7 μg/kg body weight by the Scientific Committee on Food of the European Union[13]. Recently, a subchronic toxicity study of NIV using F344 rats was conducted, and the no-observed-adverse-effect level (NOAEL) of NIV was less than 6.25 ppm (0.4 mg/kg body weight/day for both male and female rats) based on hematological changes showing decreased white blood cell counts[14]. In an extension of the subchronic study, natural killer (NK) activity against YAC-1 target cells by lymphocytes from the spleen derived from the subchronic toxicity study increased in males *in vitro* [15], indicating that orally administered NIV is immunotoxic. In young pigs, oral administration of NIV for 3 weeks showed toxicities in the gastrointestinal tract and kidneys and reduced the number of splenocytes[16]. Additionally, the same study found that 2.5 mg/kg NIV caused a time-dependent

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*Corresponding author: K Inoue (e-mail : k-inoue@nihs.go.jp)

increase in the plasma IgA concentration.

The kidney is known to be a major target of NIV toxicity. Mice orally administered NIV for up to 8 weeks showed increased serum IgA concentrations and histopathological changes in the renal glomeruli such as mild mesangial expansion[17]. In another study, 24 ppm NIV administered orally for up to 8 weeks increased serum IgA levels and glomerular deposition of IgA and IgG in a dose-dependent manner in female BALB/c mice and also increased the serum IgA level in a high IgA strain (HIGA) of mice, an animal model for human IgA nephropathy[18]. According to the previous reports, oral administration of NIV or trichothecene vomitoxin to mice increased IgA(+) B cells or IgA production from Peyer's patches or splenocyte cultures, respectively[19, 20], which might cause elevation of the serum IgA concentration and IgA deposition in the glomeruli in mice. Although many studies have reported renal toxicities of NIV, there is limited toxicological data regarding renal toxicity in children with or without renal disease.

Since there are reports that NIV increases the serum IgA concentration and deposition of IgA in the glomerular mesangial areas, there is a possibility that NIV would aggravate or modify developed glomerular lesions, especially in infant animals, which might be more sensitive to toxic chemicals. ICGN mice are an inbred strain with hereditary nephrotic syndrome and they are considered a good animal model of human idiopathic nephrotic syndrome[21–23]. Since early onset of glomerular alteration and proteinuria were observed in the neonatal or infant period, 3-week-old ICGN mice were selected as a model of human infant patients with renal disease to clarify whether NIV aggravated the nephritic syndrome and renal lesions observed in infant ICGN mice. In addition, infant ICR mice, the genetic background of ICGN mice, were used as a model for healthy human infants. We focused on the effect of NIV on the renal glomeruli in 3-week-old ICGN and ICR mice, and changes in serum biochemistry, histopathology, and immunohistopathology of the kidneys were analyzed and compared with non-treated control animals or 8-week-old adult ICR mice.

Materials and Methods

Chemicals

For purification of NIV, Fusarenon X was extracted and purified from the cultured media of *Fusarium kyushuense* (Fn-2B). Purified NIV was mixed with the basal diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) for animal studies, and NIV was extracted from the basal diet and analyzed. The identity and purity of NIV was determined by liquid chromatography/mass spectrometry (LC/MS; LCMS-2010A; Shimadzu Corp., Kyoto, Japan), with an atmospheric pressure chemical ionization interface and LC system (LC-2010CHT; Shimadzu Corp.), and the purity was estimated to be >90% from the area percentage of the chromatogram (data not shown).

Dietary treatment of NIV was chosen because it is the same exposure route as for humans. NIV was dissolved in a small quantity of ethanol and was then mixed well with powdered CRF-1 basal diet to obtain the 6, 12 and 24 ppm doses. The stability of NIV in the basal diet at each dose was analyzed immediately and 2 weeks after food preparation to examine the effect of storage condition at room temperature or 4°C. Since the stability of NIV in the diet decreased under all preservation conditions, the final concentration of the prepared diet were calculated to be 4, 8, and 16 ppm.

Animals

ICGN mice were bred in the National Institute of Health Sciences, Japan. Male and female ICGN/M mice showing proteinuria were mated at the age of 8 weeks old, and their 3-week-old male offspring were used in the present study. Neonatal ICGN mice were underdeveloped due to nephritic syndrome in their dams or their own renal problems. For comparison with ICR mice at the same age or with adults, 2-week-old and 7-week-old male Slc:ICR mice were purchased from Japan SLC, Inc. (Shizuoka, Japan), and infant ICR mice were maintained with their dams until being weaned. Mice were housed 5 or 6 per polycarbonate cage with sterilized softwood chips as bedding in a barrier-sustained animal room maintained at 23–25°C and 50–60% humidity with a 12 h light/dark cycle.

Experimental design

ICGN and young and adult ICR mice were randomly divided into 4 groups. Each group consisted of 6 ICGN mice or 10 ICR mice. Due to the difficulty of obtaining neonatal ICGN mice, the number of ICGN mice used in the present study was lower than the number of ICR mice. Animals were administered 0 (control), 4, 8, or 16 ppm NIV mixed in powdered diet for 4 weeks. The diet and drinking water were given *ad libitum*. The animals were checked daily for clinical signs and mortality. Body weights were measured weekly, and all mice were checked weekly for proteinurea using test strips (Uropaper III *Eiken*, Eiken Chemical Co., Ltd., Tochigi, Japan). Food intake was weighed weekly, and the NIV intake was calculated. At the end of the experiment, all of the animals were deeply anesthetized, blood samples were collected from the abdominal vein, and the animals were euthanized. The serum, obtained from centrifugation of the blood at 3,000 ppm, was stored until serum biochemical analysis. The left and right kidneys were removed, weighed and sectioned to provide central slices including the pelvis, and the slices were fixed with 10% neutral buffered formalin for histopathological and immunohistochemical examination. The remainder of the kidney tissue was fresh frozen with liquid nitrogen and stored at –80˚C until used for immunofluorescent assessment.

The animal protocol was reviewed and approved by the Animal Care and Use Committee of the National Institute of Health Sciences, Japan.

Analysis of serum biochemistry and IgA concentration

To examine the condition of nephritic syndrome and renal function, serum concentrations of total protein (TP), albumin (Alb), total cholesterol (TC), blood urea nitrogen (BUN), and creatinine (CRN) were analyzed (SRL, Inc., Tokyo, Japan).

An enzyme-linked immunosorbent assay (ELISA) was performed to analyze the serum IgA concentration using a Mouse IgA ELISA Quantitation Kit (Bethyl Laboratories, Inc., Montgomery, TX, USA) following the manufacturer's protocol. In brief, after coating wells with goat anti-mouse IgA-affinity purified antibody and blocking solution, diluted mouse reference serum standards and samples were incubated at room temperature for 60 minutes. The dilution ratios for serum samples were 500 and 2,500 for ICGN and ICR mice, respectively. Goat anti-mouse IgA antibody conjugated with horseradish peroxidase was then incubated in each well, and the enzyme substrate reaction was performed with tetramethylbenzidine (TMB) solution. To stop the TMB reaction, 2 M sulfuric acid was added to the wells, and the absorbance was measured using a multichannel automatic photometer (Multiskan MS; Labsystems, Helsinki, Finland).

Histopathological assessment

The kidney slices fixed in 10% buffered formalin were routinely embedded in paraffin and were stained with hematoxylin and eosin and Periodic acid-Schiff (PAS) stains. To detect damage of mesangial areas by NIV, the number of glomeruli showing mesangial expansion was counted in the cross sections for bilateral kidneys using PAS sections, and the percentage of affected glomeruli per animal was calculated.

Immunohistochemical and immunofluorescent assessment

Since mesangial expansion was observed in the glomeruli of ICGN mice, proliferative and activated mesangial cells were evaluated by immunohistochemical analysis. Formalin-fixed, paraffin-embedded renal sections were treated with 0.3% H_2O_2 in absolute methanol after heating in instant antigen-retrieval agent H (neutral) (Mitsubishi Chemical Medience Corporation, Tokyo, Japan) at 90˚C for 10 minutes. The sections were incubated with a mouse anti-human smooth muscle actin (α-SMA) monoclonal antibody (clone 1A4, ×100 dilution; Dako Japan, Tokyo, Japan), a marker of activated mesangial cells, or anti-proliferating cell nuclear antigen (PCNA) monoclonal antibody (clone PC10, ×100 dilution; Dako Japan, Tokyo, Japan) at 4°C overnight. Immunodetection was carried out with Histofine® Simple Stain MAX PO (MULTI) (Nichirei Biosciences Inc., Tokyo, Japan) and was visualized with 3,3'-diaminobenzidine as the chromogen. To evaluate activated mesangial cells, the number of glomeruli showing α-SMA-positive mesangial areas was counted in the bilateral kidneys (81–124 glomeruli in ICGN mice and 130–231 glomeruli in 8-week-old ICR mice), and the ratios of glomeruli with α-SMA-positive mesangial areas to all glomeruli were analyzed.

To detect IgA deposition in the glomeruli, immunofluorescent assessment was performed. Frozen kidney tissues were embedded with Tissue-Tek® O.C.T. compound (Sakura Finetek Japan Co. Ltd., Tokyo, Japan), and 5 μm-thick frozen tissue sections were prepared. The frozen sections were air-dried and fixed with cold acetone for 10 minutes. After fixation, the sections were again air-dried and preserved at –30°C until use. After air-drying and washing with PBS, goat anti-mouse IgA (a-chain specific) antibody (Sigma, St. Louis, MO, USA) was applied to the sections, and they were incubated at room temperature for one hour. For visualization, FITC-labeled rabbit anti-goat IgG heavy and light chain specific antibody (NOVUS Biologicals, LLC, Littleton, CO, USA) was applied as a secondary antibody, and the glomeruli were observed under a fluorescence microscope (BX51, Olympus, Tokyo, Japan).

Statistical analysis

Variances in the data for body and kidney weight, serum biochemistry, serum IgA concentration, level of proteinuria, and percentage of glomeruli with α-SMA-positive mesangial cells were checked for homogeneity by Bartlett's procedure. If the variance was homogeneous, the data were assessed by one-way analysis of variance. If not, the Kruskal-Wallis test was applied. When statistically significant differences were indicated, the Dunnett multiple comparison test was employed for comparison between the control and treatment groups. For histopathological changes, incidences were compared using the Fisher exact probability test, and severity was analyzed with the Mann-Whitney *U*-test.

Results

Final body and kidney weights are shown in Table 1. ICGN mice had lower final body weights and absolute kidney weights than ICR mice at the same age. For ICGN mice, no significant differences in final body weight and kidney weight between control and treated groups were observed. In the 16 ppm NIV group of ICGN mice, a tendency towards a decreased final body weight was observed. For ICR mice, there was a significant decrease in final body weight in the infant 16 ppm NIV group, while there was no significant difference between controls and NIV-treated groups in adult ICR mice.

Mean food consumption and intake of NIV are shown in Table 2. Mean daily food consumption decreased in the 16 ppm NIV groups for each strain and generation. Mean daily NIV intake almost doubled within strains and generations of mice as the dose increased.

In the serum biochemistry, significant decreases in blood urea nitrogen (BUN) levels in the 16 ppm group of ICGN mice and in creatinine levels (CRN) in the 16 ppm group of infant ICR mice and 8 ppm group of adult ICR mice were observed compared with the corresponding con-

trols (Table 3). The serum biochemical data of the ICGN mice did not show significant differences from controls or infant ICR mice. Other serum biochemistry parameters did not show any significant changes except for an increase in Alb in the 16 ppm NIV group of adult ICR mice. The con-

Table 1. Final Body and Absolute and Relative Kidney Weights of ICGN and ICR Mice Treated with NIV for 4 Weeks

Group	No. of animals examined	Final BW (g)	Kidneys	
			Absolute (g)	Relative (g/100 g BW)
ICGN (3 wks)				
Control	6	21.4 ± 0.8[a)]	0.33 ± 0.12	1.54 ± 0.12
4 ppm NIV	6	21.3 ± 2.7	0.32 ± 0.16	1.47 ± 0.16
8 ppm NIV	6	20.5 ± 2.3	0.30 ± 0.19	1.46 ± 0.19
16 ppm NIV	6	19.3 ± 2.3	0.28 ± 0.11	1.45 ± 0.11
ICR (3 wks)				
Control	10	39.5 ± 3.0	0.55 ± 0.06	1.41 ± 0.13
4 ppm NIV	10	38.7 ± 2.6	0.54 ± 0.09	1.38 ± 0.20
8 ppm NIV	10	38.0 ± 3.0	0.55 ± 0.07	1.46 ± 0.22
16 ppm NIV	10	35.8 ± 2.0*	0.53 ± 0.05	1.48 ± 0.11
ICR (8 wks)				
Control	10	46.3 ± 3.3	0.68 ± 0.05	1.47 ± 0.15
4 ppm NIV	10	44.2 ± 4.4	0.68 ± 0.07	1.56 ± 0.23
8 ppm NIV	10	46.2 ± 2.6	0.69 ± 0.08	1.49 ± 0.16
16 ppm NIV	10	45.7 ± 1.7	0.63 ± 0.04	1.39 ± 0.09

* Significantly different from controls at $p<0.05$. [a)] Mean ± SD.

Table 2. Mean Food Consumption and Intake of NIV in ICGN and ICR Mice

Group	Mean food consumption (g/animal/day)[a]	Mean daily intake of NIV (mg/kg BW/day)[b]	Total intake of NIV (mg/kg bw)[c]
ICGN (3 wks)			
Control	2.4	0	0
4 ppm NIV	2.2	0.37	10.4
8 ppm NIV	2.1	0.70	19.5
16 ppm NIV	2.0	1.39	39.0
ICR (3 wks)			
Control	4.71	0	0
4 ppm NIV	4.56	0.58	16.2
8 ppm NIV	4.54	1.18	33.1
16 ppm NIV	4.05	2.25	63.1
ICR (8 wks)			
Control	5.18	0	0
4 ppm NIV	5.30	0.49	13.8
8 ppm NIV	5.22	0.96	26.8
16 ppm NIV	4.80	1.76	49.3

[a] Average of the mean daily food intake per animal in each week. [b] Average of the mean daily NIV intake per kg body weight in each week. [c] Total value of NIV for 4 weeks calculated with the mean daily intake of NIV in each week.

Table 3. Serum Biochemistry of ICGN and ICR Mice Treated with NIV for 4 Weeks

Group	No. of animals examined	Total protein (g/dl)	Albumin (g/dl)	Total cholesterol (mg/dl)	Blood urea nitrogen (mg/dl)	Creatinine (mg/dl)
ICGN (3 wks)						
Control	6	4.62 ± 0.55[b)]	2.72 ± 0.37	138.8 ± 9.8	21.2 ± 3.9	0.082 ± 0.032
4 ppm NIV	5[a]	4.60 ± 0.32	2.92 ± 0.36	113.8 ± 17.4	17.7 ± 1.9	0.080 ± 0.007
8 ppm NIV	6	4.57 ± 0.31	2.83 ± 0.34	128.7 ± 25.1	16.9 ± 3.3	0.075 ± 0.016
16 ppm NIV	6	4.45 ± 0.29	2.75 ± 0.41	133.0 ± 29.7	15.7 ± 2.7*	0.070 ± 0.006
ICR (3 wks)						
Control	10	4.76 ± 0.20	2.82 ± 0.14	152.0 ± 23.0	24.6 ± 4.2	0.085 ± 0.012
4 ppm NIV	10	4.73 ± 0.11	2.86 ± 0.11	145.3 ± 21.7	25.1 ± 3.4	0.084 ± 0.005
8 ppm NIV	10	4.77 ± 0.24	2.90 ± 0.12	143.3 ± 14.6	22.7 ± 2.1	0.076 ± 0.011
16 ppm NIV	10	4.62 ± 0.34	2.90 ± 0.20	137.5 ± 13.4	22.8 ± 2.9	0.066 ± 0.014**
ICR (8 wks)						
Control	10	5.04 ± 0.36	2.83 ± 0.20	140.6 ± 33.4	23.1 ± 2.8	0.085 ± 0.014
4 ppm NIV	10	4.85 ± 0.20	2.81 ± 0.14	127.9 ± 24.3	25.4 ± 4.1	0.080 ± 0.011
8 ppm NIV	10	4.98 ± 0.20	2.87 ± 0.16	158.5 ± 30.1	24.0 ± 2.1	0.071 ± 0.012*
16 ppm NIV	10	5.00 ± 0.27	3.06 ± 0.13**	146.2 ± 22.3	24.2 ± 2.7	0.075 ± 0.008

[a] One animal could not be measured because of an insufficient of serum volume. [*, **] Singificantly different from controls of each animal at $p<0.05$ and $p<0.01$, respectively. [b)] Mean ± SD.

Table 4. Serum IgA Concentration (mg/ml) in ICGN and ICR Mice Treated with NIV for 4 Weeks

Group (n=)	Control	4 ppm NIV	8 ppm NIV	16 ppm NIV
	6 or 10	6 or 10	6 or 10	6 or 10
ICGN (3 wks)	173.1 ± 58.9[a)]	193.4 ± 75.2	213.4 ± 71.5	202.8 ± 49.3
ICR (3 wks)	325.0 ± 63.8	367.1 ± 94.0	356.2 ± 58.1	441.0 ± 106.0*
ICR (8 wks)	774.5 ± 171.5	734.0 ± 183.9	721.9 ± 346.2	681.2 ± 163.3

* Significantly different from controls at $p<0.05$. [a)] Mean ± SD.

centration of IgA in the serum tended to increase, but there was no significant difference between controls and NIV-treated groups in ICGN mice (Table 4). In infant ICR mice, the serum IgA concentration in the 16 ppm group increased significantly, while there were no changes after NIV treatment in adult ICR mice.

In all of the ICGN mice, the levels of proteinuria were higher than in age-matched ICR mice, indicating onset of proteinuria in infant ICGN mice (Fig. 1). However, the levels of proteinuria did not rise with NIV treatment in any strain or generation.

Histopathologically, a thickened basement membrane, mesangial expansion, and microaneurysm were observed in the glomeruli of all ICGN mice groups including controls (Fig. 2). The thickened glomerular basement membrane was observed diffusely in the kidneys of ICGN mice. The

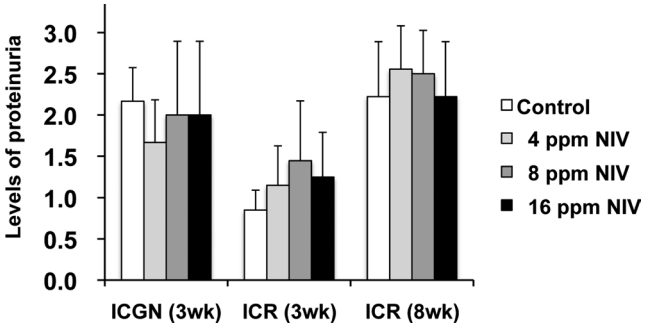

Fig. 1. Levels of proteinuria in ICGN and ICR mice treated with NIV for 4 weeks (Mean ± SD). The levels of proteinuria did not rise with NIV treatment in any strain or generation. NIV, nivalenol.

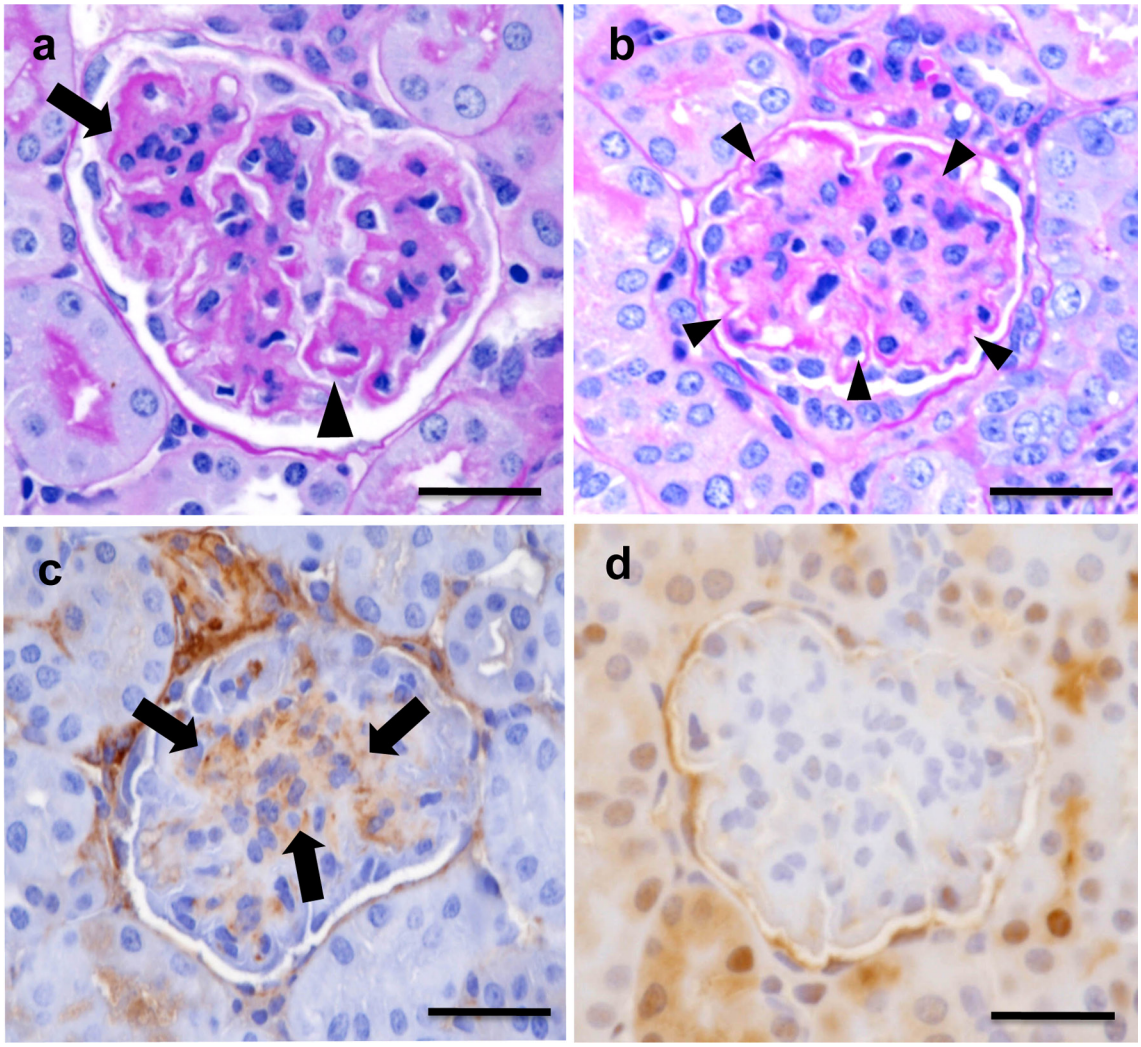

Fig. 2. Histopathological and immunohistochemical findings in the glomeruli of ICGN mice treated with 16 ppm NIV for 4 weeks. Genetic glomerular lesions known in ICGN mice were observed as follows: (a) Diffuse thickened glomerular basement membrane (arrowhead) and segmental (arrow) or (b) diffuse mesangial expansion were observed (arrowheads; PAS). (c) α-SMA-positive mesangial cells (arrows) were observed in the expanded mesangial area. (d) Mesangial cells in the expanded mesangial area were negative for PCNA. (c) and (d) The mesangial cells in NIV-treated infant ICGN mice were activated but not proliferative. Bars = 25 µm.

number of glomeruli with mesangial expansion tended to increase in NIV-treated groups, but there was no statistically significant difference (Fig. 3a). Microaneurysms in the glomeruli and urinary casts in the distal tubules were also observed, but NIV treatment did not enhance these lesions in ICGN mice (data not shown).

In ICGN mice, mesangial cells of the expanded mesangial area were positive for α-SMA (Fig. 2c), and the number of glomeruli with α-SMA-positive mesangial cells increased in the 16 ppm NIV group without statistical significance (Table 5). In the expanded mesangial area of ICGN mice, PCNA-positive mesangial cells were not observed in any of the glomeruli (Fig. 2d).

Mild to severe mesangial expansion was observed in 1.21% of the total glomeruli of the bilateral renal tissue sections in the 16 ppm NIV group of adult ICR mice (Fig. 4d) compared to 0.23% in the control group (Fig. 3b). No change in the mesangium was observed in lower-dose adult groups or any treated infant ICR mouse groups (Figs. 4a, b, and c).

A small amount of granular deposition of IgA in the glomeruli was detected at the glomerular basement membrane and mesangial area in all of the ICGN mouse groups including controls. The level and localization of IgA deposition did not show any differences between the control and NIV-treated groups (Fig. 5). In infant and adult ICR mice, IgA deposition was observed in the glomerular basement membrane and mesangial area in all groups including controls (Fig. 6). The level and localization of IgA deposition were almost the same regardless of age or NIV-treatment. Compared with ICGN mice, the level of glomerular IgA deposition was lower in infant ICR mice at the same age.

Discussion

In the present study, the effects of NIV on the kidneys of infant mice were evaluated, and some changes were detected in ICGN mice. In adult ICGN mice, genetic glomerular lesions including a thickened glomerular basement membrane, focal or diffuse mesangial expansion without mesangial cell proliferation and microaneurysm were observed. Since 12 ppm NIV is known to induce IgA deposition in the mesangial area in normal C3H/HeN and C3H/HeJ mice[17], it would be expected that IgA deposition by NIV might induce additional mesangial lesions such as mesangial damage/proliferation and progressive matrix production in the mesangial area of ICGN mice. However, such noticeable effects were not detected in the glomeruli of NIV-treated infant ICGN mice in the present study, except for an increased number of glomeruli with α-SMA-positive mesangial cells. Four weeks of treatment with 24 ppm NIV was reported to increase the serum IgA level and IgA deposition in the glomeruli of female BALB/c mice, yet the same treatment did not enhance immunoglobulin deposition in the glomeruli of high IgA strain (HIGA) mice[18]. However, NIV did not induce or enhance glomerular lesions, such as mesangial expansion, in either BALB/c or HIGA mice in the reported study. It was also reported that deoxynivalenol, another trichothecene mycotoxin, increased levels of serum IgA, circulating IgA immune complexes, mesangial IgA deposition, and hematuria without significant mesangial lesions in mice[20, 24, 25]. Based on the results of the present and

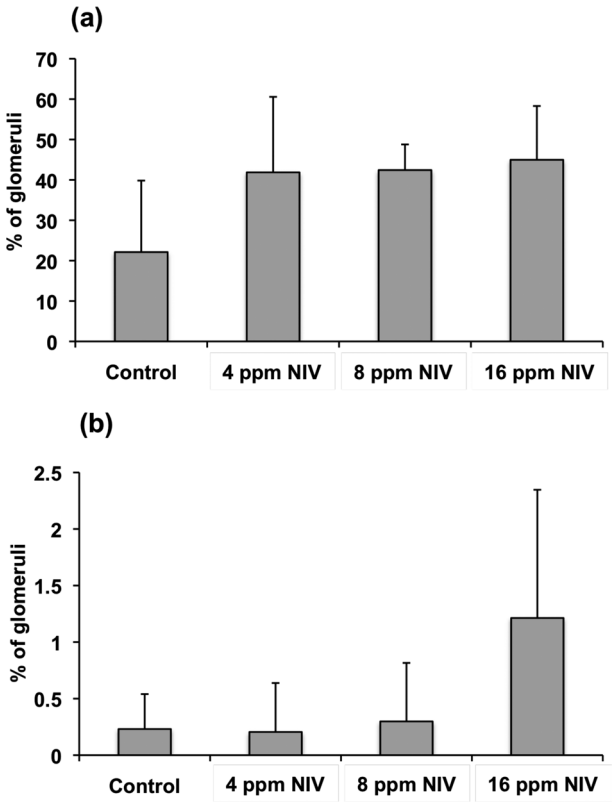

Fig. 3. Percentages of glomeruli with expanded mesangial areas and α-SMA-positive mesangial cells in the kidneys (Mean ± SD). (a) The number of glomeruli with mesangial expansion tended to increase without statistical significance in ICGN mice. (b) In adult ICR mice, mild to severe mesangial expansion was observed in 1.21% of the total glomeruli of the bilateral renal tissue sections in the 16 ppm NIV group compared with 0.23% in the control group. Expanded mesangial area in the glomeruli was not observed in infant ICR mice.

Table 5. Percentage of Glomeruli with α-SMA-positive Mesangial Cells in ICGN Mice Treated with Nivalenol for 4 Weeks

Group	Control	4 ppm NIV	8 ppm NIV	16 ppm NIV
No. of animals examined	6	6	6	6
% of glomeruli with α-SMA-positive mesangial cells	33.2±12.4[a]	32.3±14.2	28.8±11.9	41.1±21.4

[a] Mean ± SD.

reported studies, treatment with NIV for 4 weeks might be insufficient to induce aggravation of genetic glomerular lesions or obvious histopathological changes in the glomeruli and other renal components in ICGN mice. In particular, the highest dose of NIV was 16 ppm in the present study, which was lower than the 24 ppm dose used in previous studies. Therefore, the doses in the present study might be insufficient to induce or exacerbate glomerular lesions in ICGN mice.

In the present study (Table 6), NIV treatment with ICR mice led to increases in the serum IgA concentration in infants and mesangial expansion in adults in the 16 ppm groups. Unexpectedly, no glomerular changes were observed in infant animals compared with adult animals. This result indicates that the histopathological sensitivity to NIV might be weaker in infant mice than in adult animals. However, in adults, the percentage of glomeruli with mesangial expansion was very low (1.21%). Similar to C3H/HeN and C3H/HeJ mice[17], the observed effects of NIV in adult ICR mice in the present study were mild. Therefore, the effect of NIV on the kidneys of adult ICR mice might be generally mild. In addition, it was reported that the sensitivity of kidneys to toxic chemicals shows strain differences. Treatment with 10 mg/kg body weight of Adriamycin (ADR) induced severe proteinuria in BALB/c mice, while the same dose of ADR did not induce proteinuria in C57BL/6 mice[26]. Therefore, ICR mice could potentially be a resistant strain to the effect of NIV. A further factor regarding weak NIV effects in ICR mice may be the NIV concentration in the diet. Although the cause of the decreased concentration of NIV in the prepared diet could not be identified, the variation of NIV concentration in the diet might also be a cause for reduced NIV toxicity.

In humans, IgA nephropathy (IgAN) is defined as chronic glomerulonephritis accompanied with mesangial proliferation, expansion of the extracellular matrix, and granular IgA deposition in the mesangial area. Recently, it was shown that circulating immune complexes contain-

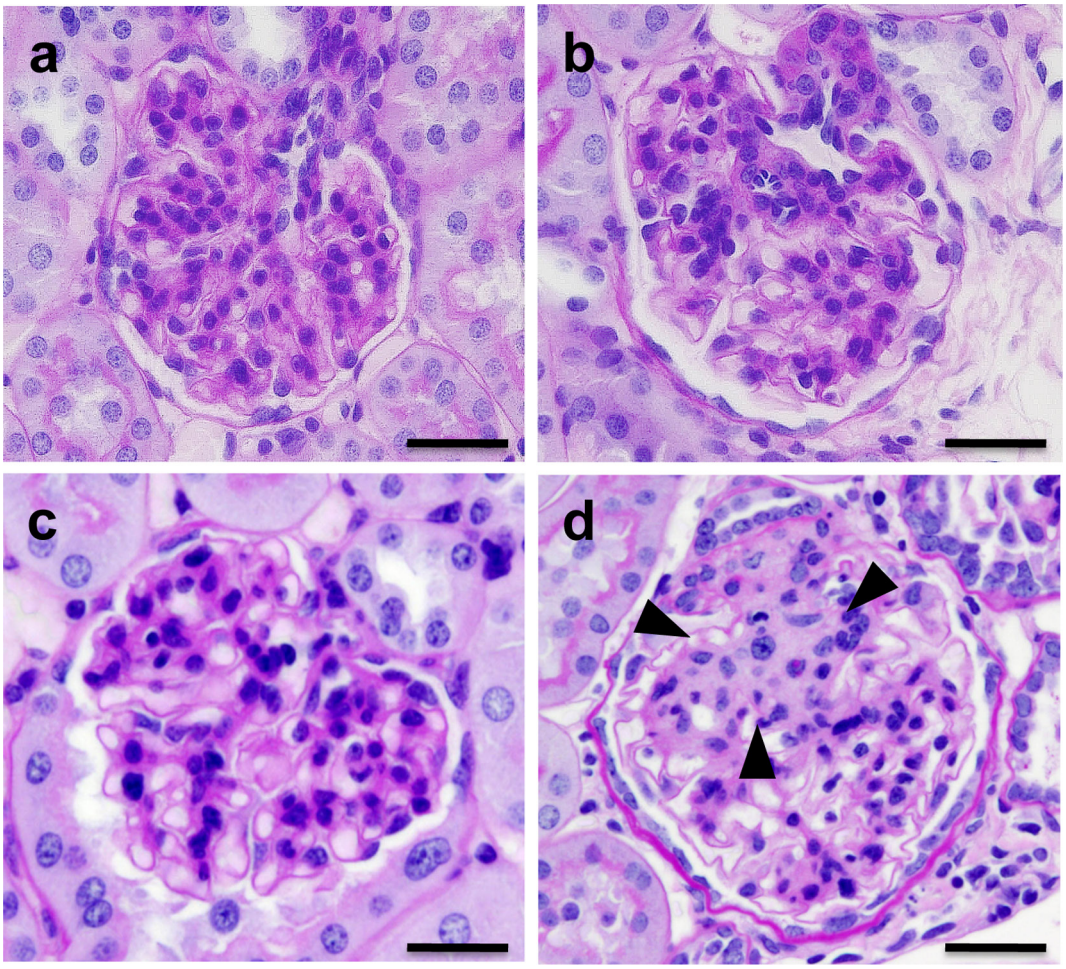

Fig. 4. Histopathological features in the glomeruli of ICR mice treated with nivalenol for 4 weeks. (a) Control, 3-week-old group; (b) 16 ppm NIV, 3-week-old group; (c) control, 8-week-old group; and (d) 16 ppm NIV, 8-week-old group. Mesangial expansion (arrowheads in (d)) was observed in a glomerulus of the bilateral renal tissue sections in the 16 ppm NIV group of adult ICR mice. On the other hand, the change in the mesangium was not observed in NIV-treated infant ICR mice. Bars = 25 µm.

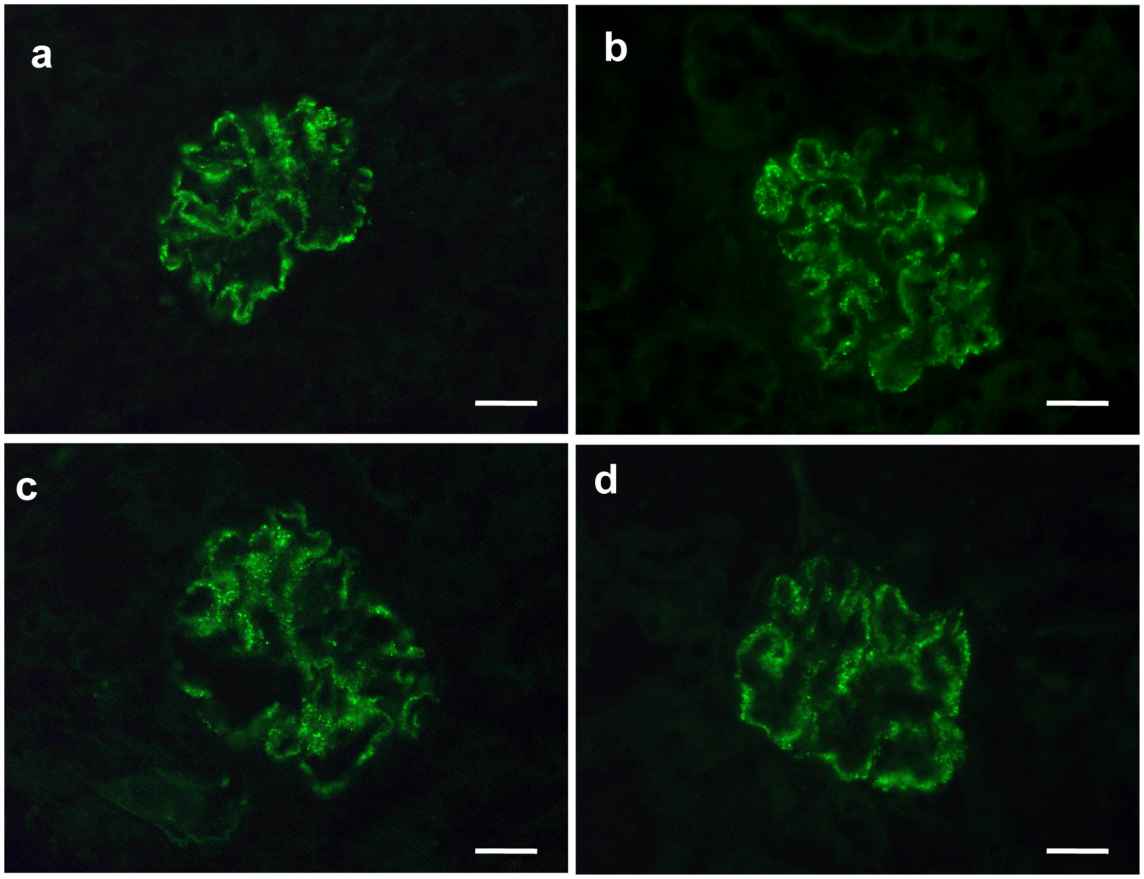

Fig. 5.

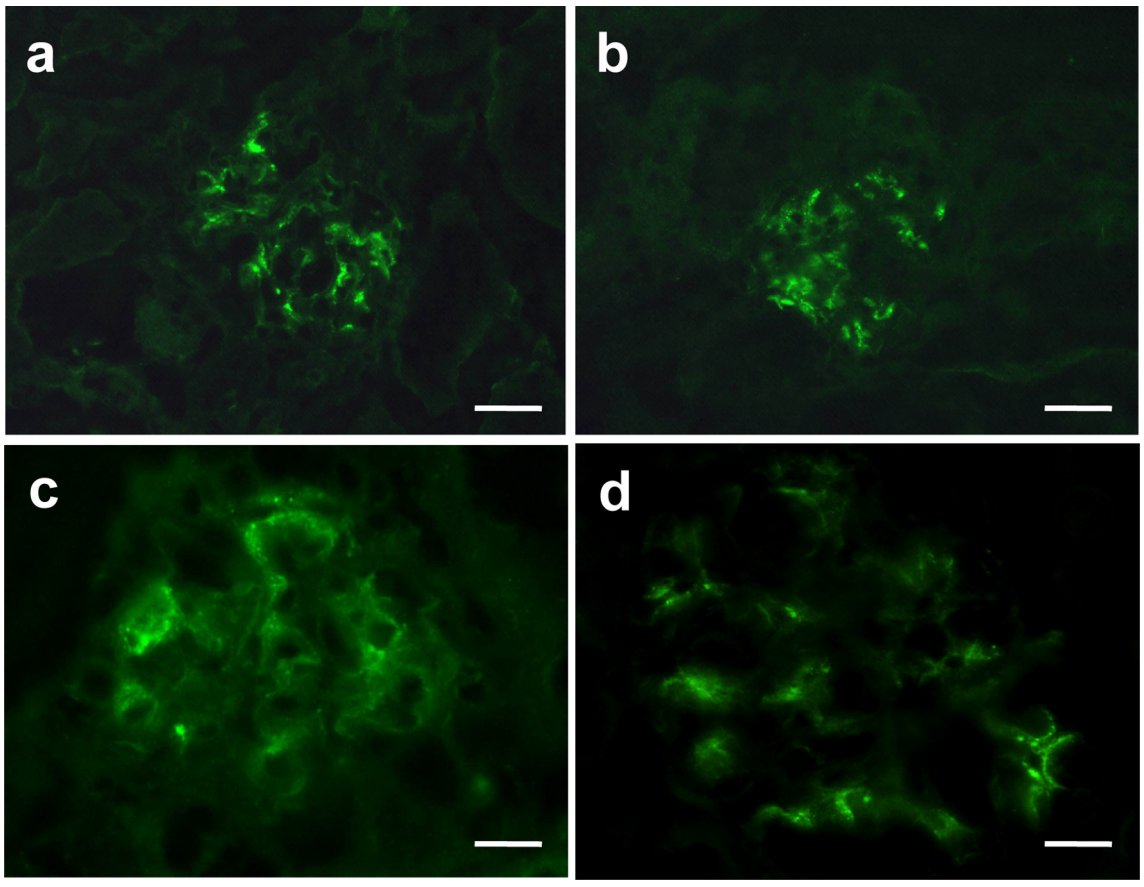

Fig. 6.

Fig. 5. IgA deposition in the glomeruli of ICGN mice treated with nivalenol for 4 weeks. (a) Control, (b) 4 ppm NIV, (c) 8 ppm NIV and (d) 16 ppm NIV groups. The level and localization (in the glomerular basement membrane and mesangial area) of IgA deposition did not show any differences between control and NIV-treated groups. NIV, nivalenol. Bars = 25 µm.

Fig. 6. Representative findings of IgA deposition in the glomeruli of ICR mice treated with nivalenol for 4 weeks. (a) Control, 3-week-old group; (b) 16 ppm NIV, 3-week-old group; (c) Control, 8-week-old group; and (d) 16 ppm NIV, 8-week-old group. The IgA deposition level and localization (in the glomerular basement membrane and mesangial area) were almost the same, regardless of age or NIV-treatment. Compared with ICGN mice, the level of glomerular IgA deposition was lower in infant ICR mice. NIV, nivalenol. Bars = 25 µm.

Table 6. Summary of Changes Induced with 16 ppm NIV Treatment in ICGN and ICR Mice

Endopoints of effects on the glomeruli	Strain (age)		
	ICGN (3 wks)	ICR (3 wks)	ICR (8 wks)
Serum IgA concentration	—	↑	—
Proteinuria	—	—	—
Enhancement of genetic glomerular lesions	—	N.A.	N.A.
No. of glomeruli with α-SMA-positive mesangium	↑	N.A.	N.A.
Incidence of mesangial expansion	N.A.	—	↑
IgA deposition in the glomeruli	—	—	—

–: No change was observed compared with the corresponding controls. ↑: NIV increased the paramater with or without significance. N.A.: Not analyzed.

ing aberrantly glycosylated IgA1 play a pivotal role in the pathogenesis of human IgAN[27]. Although an increase in serum IgA concentration in infant mice and deposition of IgA in the glomeruli of all treated mice were observed in the present study, mesangial lesions were not prominent. Circulating IgA without aberrant glycosylation and insufficient deposition of IgA to the mesangial area might be related to the lower incidence and severity of mesangial lesions observed in NIV-treated mice.

In conclusion, detailed analyses of the glomeruli did not provide clear evidence that diseased or healthy infant mice were toxicologically sensitive to NIV under the present experimental conditions. Human infants face a risk of exposure to nephrotoxins in their daily food intake, including mycotoxins. Further studies are needed to investigate the validity of the ICGN mouse model and to develop other suitable animal models for infant renal toxicity studies.

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