MEDICAL SCIENCE MONITOR

ANIMAL STUDY

e-ISSN 1643-3750
© Med Sci Monit, 2017; 23: 3170-3177
DOI: 10.12659/MSM.905073

Received: 2017.04.27
Accepted: 2017.05.25
Published: 2017.06.29

Effect of Vitamin D and Tacrolimus Combination Therapy on IgA Nephropathy

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABD Dong Yuan
BCD Zhan Fang
CDE Fang Sun
EF Jing Chang
DEF Jian Teng
FG Shuhua Lin
ACF Xiaoming Liu

Department of Nephrology, Yantaishan Hospital, Yantai, Shandong, P.R. China

Corresponding Author: Xiaoming Liu; e-mail: xiaomingliulium@163.com
Source of support: Departmental sources

Background: To explore the effects and the mechanism of vitamin D (VD) and tacrolimus (TAC) combinatorial therapy in the treatment of IgA nephropathy (IgAN) in a rat model.

Material/Methods: IgAN rat models constructed by oral immunization with bovine serum albumin (BSA) and lipopolysaccharide (LPS) (n=30) and were treated with: saline (model group), TAC (TAC group), or TAC+VD therapy (TAC+VD group) through gavage daily for 14 days. Serum creatinine (Scr), albumin (ALB), blood urea nitrogen (BUN), and urinary protein (UAE) levels were determined. Histopathology of renal tissues was examined after hematoxylin and eosin (H&E) staining. The levels of cytokines TGF-β1, IL-5, IFN-γ, and IL-4 in serum were detected by enzyme-linked immunosorbent assay (ELISA). Changes in TLR4/NF-κB pathway were evaluated by western blot.

Results: Both TAC and TAC+VD treatment significantly restored the dysregulated Scr, ALB, BUN, and UAE levels in IgAN rats. TAC+VD therapy more prominently restored Scr and UAE levels ($p<0.05$). TAC+VD therapy demonstrated superior efficacy in reducing glomerular mesangial cells hyperplasia, reducing thickening of the glomerular basement membrane and glomerular infiltration of inflammatory cells. Thymus and spleen indexes were also increased ($p<0.05$). The levels of TGF-β1, IL-5, and IL-4 of the TAC+VD group were also lower than those of the TAC group ($p<0.05$). The TAC+VD group also demonstrated increased IFN-γ, and decreased p-P65/P65 and TLR4 compared to the TAC group.

Conclusions: TAC+VD combinatorial therapy can effectively alleviate renal tissue damage in IgAN rats by regulating immune response and the NF-κB/TLR4 pathway.

MeSH Keywords: Calcitriol • Glomerulonephritis, IGA • Tacrolimus

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/905073

2674 — 5 26

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

IgA nephropathy (IgAN), also known as Berger's disease, is the most common form of glomerulonephritis [1]. IgAN is characterized by mesangiopathic glomerulonephritis with macroscopic or microscopic hematuria [1]. This disease compromises the waste-filtering ability of kidney and has a high risk of progressing into glomerulosclerosis, a devastating renal disease that eventually leads to kidney failure [2]. Although the course of the disease varies among patients, early and effective treatment of IgAN is paramount for impeding the disease progression and improving quality of life. However, no curative method exists for IgAN and there is an urgent clinical need to develop novel treatment strategies to slow the disease progression, alleviate the impairment of renal tissue, and possibly restore normal renal functions.

Tacrolimus (TAC) is commonly used for the treatment of IgAN and has shown efficacy in improving remission from proteinuria in patients with refractory IgAN [3]. TAC acts as an immunosuppressant, which is exploited in patients with heart, liver, kidney, or pancreas transplantation [4]. TAC has the ability to promote T-cell activation and proliferation; it regulates the antioxidant status and protects organs from hypoxic insults. Despite the ameliorating effects of TAC in IgAN patients, satisfactory clinical outcomes have yet to be achieved, which has led to a number of efforts to combine other therapeutics to augment the protection in renal tissue [5,6].

Recent evidence indicates that vitamin D insufficiency is associated with poorer outcomes of IgAN patients [7]. Vitamin D insufficiency causes albuminuria and is not conducive to kidney injury recovery. In IgAN patients, such disorders dramatically increase the morbidity of the diseases and harm patients' life quality. Because of the important role of vitamin D in maintaining normal vascular function, vitamin D supplementation has been used as a strategy in treating IgAN and has shown effects in stabilization of cardiac autonomic tone in IgAN [8]. Moreover, vitamin D has also been shown as an important regulator of immune response [9]. However, the combination of TAC and vitamin D (TAC+VD) in IgAN has not been tested. We thus reasoned that the vascular stabilizing function and immunosuppressive property of vitamin D can potentially synergize with TAC to achieve better therapeutic outcome for IgAN patients.

Therefore, here we explored the therapeutic effects of TAC+VD combinatory therapy in alleviating renal tissue damage caused by IgAN. To this end, we constructed rat IgAN models by oral immunization. We also sought to explore the mechanism of these therapeutic effects by examining its regulation of immunological factors and the NF-κB/TLR4 pathway. The results shown in this study could potentiate TAC+VD combinatory therapy in clinical use to improve the therapeutic outcome of IgAN patients.

Material and Methods

Animal study

All animal studies were performed in accordance to regulations of the Yantaishan Hospital. Healthy Sprague-Dawley (SD) rats weighing 200–220 g were housed in a SPF-class animal room, maintained at 22–24°C. The relative humidity was kept at 50–60%. Rats were allowed to adapt to the environment for a week, during which time the rats were fed with regular chow and water autonomously. Rats in the control group were treated with 4 mL/kg of distilled water through gavage every two days for a total of eight weeks and 0.9% sodium chloride was subcutaneously injected at the dose of 0.4 mL per rat every week for nine weeks. Starting from the sixth week, sodium chloride was administered subcutaneously at the dose of 0.2 mL per rat. To construct IgAN rats, bovine serum albumin (BSA) crystal powder (Sigma, Saint Louis, MO, USA) was mixed with distilled water at the concentration of 100 g/L, which was used to treat rats at the dose of 4 mL/kg through gavage every two days for eight weeks; chloroform (CCl_4) and castor oil were mixed at a ratio of 1: 3 and used to treat rats at the dose of 0.4 mL per rat by subcutaneous injection once a week for nine weeks. Lipopolysaccharide (LPS) (Sigma) was mixed with 0.9% sodium chloride to the concentration of 0.25 g/L and was used to treat rats at the sixth week by intravenous injection of 0.2 mL per rat. TAC was acquired from the Astellas Ireland Co. Ltd. Thirty IgAN rats were randomly divided into three groups (n=10 per group) and received treatment of TAC (TAC group), TAC plus vitamin D (TAC+VD group). The model group was given 0.9% saline at the dose of 0.2 mL per day. The TAC group received treatment of TAC (4 mg/kg/day); vitamin D was dissolved in 0.5 mL peanut oil and TAC+VD group received intragastric administration of TAC (4 mg/kg/day) and vitamin D (0.03 μg/kg/day). The initial administration time was recorded as the first day and treatment continued for 14 days.

Blood and urine sample analysis

After 14 days of continuous treatment, urine was collected using metabolic cage for 24 hours. Anesthesia by intraperitoneal injection of 2% sodium pentobarbital was performed; blood was taken from the eye using a capillary glass tube. The spleen was collected; and the kidneys were taken in 4% neutral formaldehyde solution and snap-frozen for pathological analysis. Blood collected from the eye (2–3 mL) was analyzed for serum creatinine (SCr), albumin (ALB), and blood urea nitrogen (BUN) levels. Urinal total protein content was also measured. The serum levels of TGF-β1, IL-5, IFN-γ, and IL-4 were measured by ELISA. The levels of cytokines TGF-β1, IL-5, IFN-γ, and IL-4 in serum were measured by ELISA (Abcam, USA) according to manufacturer's recommendations. The LifeScan OneTouch

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

SureStep microplate reader (LifeScan, USA) was used to determine level of biochemical parameters.

Histopathological examination

The renal tissue was fixed in 4% neutral formaldehyde solution for 24 hours, dehydrated, embedded in paraffin, and sliced at the thickness of 5 microns. For H&E staining, tissue slices underwent standard rehydration and deparaffinization procedures before being stained with Mayer's hematoxylin (Abcam, USA). Images of the stained tissue were taken under a light microscope equipped with a camera (Olympus BX51, Japan).

Thymus and spleen index determination

After 14 days of continuous treatment, rats were sacrificed and the body weight was weighed. The weight of the thymus and spleen were recorded. The thymus/spleen index was calculated as the ratio of the weight of thymus/spleen and body weight.

Western blot

Western blotting was used to detect the expression of p-P65, P65, and TLR4 protein in rat kidney. BCA assay was used to determine protein concentration (Pierce, USA). 20 μg samples were loaded into precast gels (Abcam). SDS-PAGE electrophoresis was performed using the Mini-Protean-3 apparatus (Bio-Rad, USA). Proteins were then transferred to PVDF membranes. Non-fat milk (50 g/L) was used to block the membrane at 4°C overnight. Primary antibodies diluted with BSA (50 g/L) at the dilution of 1: 100 were used to incubate the membrane. TBST (TBS with 1 mL/L Tween-20) was used for washing three times, six minutes each time; HRP-conjugated secondary antibody (1: 5,000) was then used to incubate the membranes at room temperature for 2 hours, followed by adding chemiluminescent substrate and then imaging with the Fluorochem900-50 imaging system (Alpha Innotech, USA). Protein expression levels were normalized to β-actin. Rabbit anti-mouse p-P65, P65, and TLR43 polyclonal antibodies were purchased from Calbiochem (Germany). Goat anti-rabbit secondary antibodies were purchased from Wuhan Bude Biotechnology Co., Ltd.

Statistical analysis

SPSS19.0 statistical software was used to analyze the variance of data. All the data were expressed as mean ± standard deviation (±SD). The Student's *t*-test was used to compare the data between two groups. One-way ANOVA was used to analyze data from multiple groups. Differences with $p<0.05$ were considered statistically significant.

Results

Effects of TAC+VD on biochemical indexes in rats

We first constructed IgAN rats through oral immunization with BSA and LPS, which were then subjected to treatment with TAC or TAC+VD. Healthy rats and IgA rats treated with saline were used as controls. Treatment continued 14 days, after which the Scr, ALB, BUN, and UAE in urine were measured with ELISA. As shown in Figure 1, compared with the control group, the model group demonstrated higher Scr, BUN, and UAE levels and lower ALB levels, suggesting the hampered waster-filtering ability of the kidney. This indicated the successful construction of an IgAN rat model. Both TAC and TAC+VD treatments were able to significantly lower the levels of Scr and UAE in IgAN rats ($p<0.05$). Particularly, the levels of Scr and UAE in the TAC+VD group were significantly lower than those in the TAC group ($p<0.05$), suggesting enhanced kidney function in TAC+VD treated rats. Interestingly, there was no significant difference between the TAC group and the TAC+VD group in ALB and BUN levels, although both groups restored the dysregulated ALB and BUN levels as seen in IgAN rats. Taken together, TAC+VD treatment exerted the best therapeutic efficacy in improving the biomedical parameters in serum, suggesting an ameliorated kidney function.

Histopathology analysis

To further evaluate how treatments affected the physiology of kidney, we examined the histopathology of kidneys in different groups. As shown in Figure 2, in healthy rats, there were no proliferative glomerular mesangial cells and glomerular glomerulosclerosis. Renal tubular structure remained intact. No stromal hyperplasia and inflammatory cell infiltration were seen. In contrast, IgAN rats demonstrated moderate hyperplasia in glomerular mesangial cells and the mesangial matrix. Glomerular basement membrane showed segmental thickening and infiltration of a small number of inflammatory cells. This is accompanied by balloon adhesions and segmental sclerosis, capillary wall thickening, luminal stenosis, tubulointerstitial inflammatory cell infiltration, and partial renal tubular dilatation. In the TAC group, glomerular mesangial cells only demonstrated mild hyperplasia; glomerular infiltration of inflammatory cells infiltration was also limited. In the TAC+VD group, the mesangial cells showed the least hyperplasia; glomerular basement membrane was not significantly thickened and no glomerular inflammatory cell infiltration was seen. These data confirmed the higher efficacy of TAC+VD therapy in alleviating renal damages in IgAN rats.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

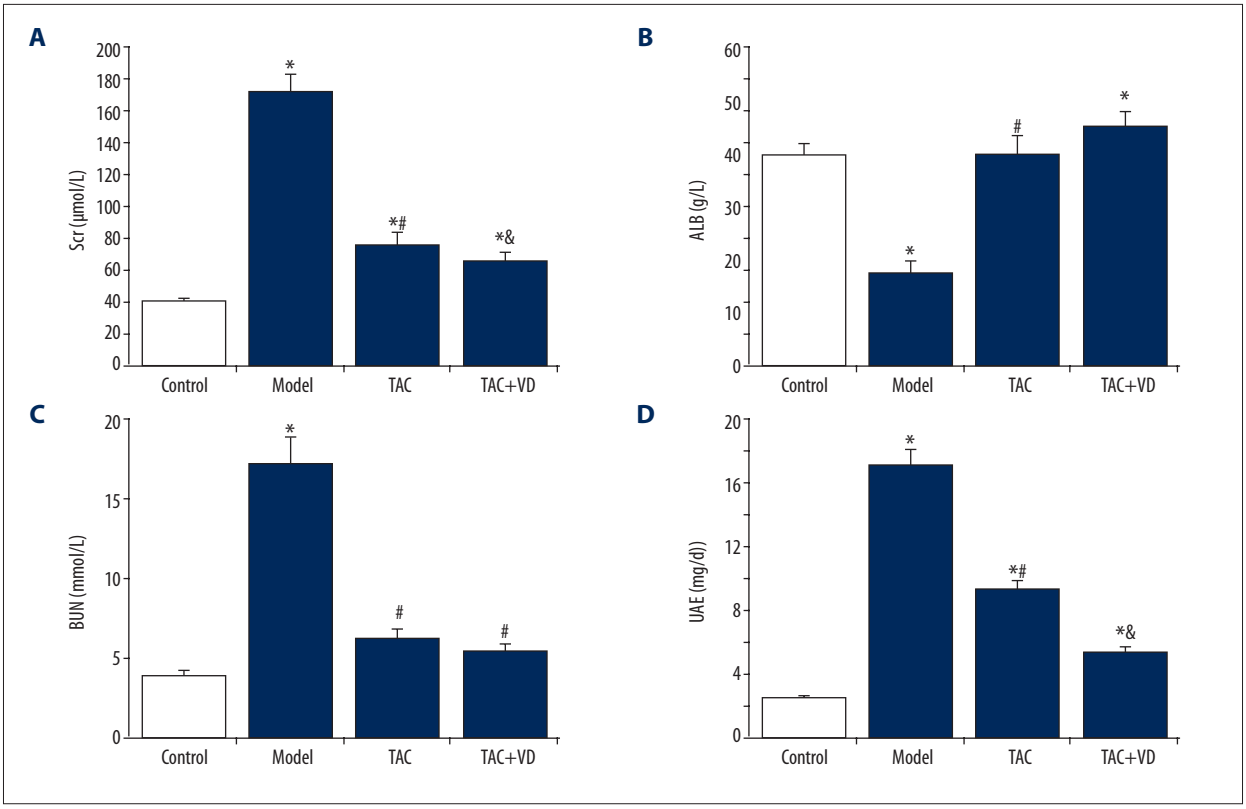

Figure 1. Levels of (**A**) Scr, (**B**) ALB, (**C**) BUN, and (**D**) UAE in rats in control, model, TAC, and TAC+VD groups; * $p<0.05$ compared with the normal group; # $p<0.05$ compared with the model group; & $p<0.05$ compared with the TAC group.

The TAC+VD combinatory therapy reduces spleen and thymus index in rats

As shown in Figure 3, compared with the control group, the thymus index of the model group and the TAC group was lower ($p<0.05$). Compared with the normal group, the TAC+VD group was significantly lower than that in the normal group ($p<0.05$). Compared with the TAC group, the thymus spleen index of the TAC+VD group was higher than that of the TAC+VD group ($p<0.05$). Spleen and thymus indexes are reflections of the immunological and inflammation levels. Therefore, TAC+VD treatment was potentially normalized immunological and inflammatory activities as reveled by improved spleen and thymus indexes.

The effect of TAC+VD on the expression of TGF-β1, IL-5, IFN-γ, and IL-4

To investigate if the ameliorating effects of TAC+VD therapy stemmed from its regulation of immunological response, the levels of TGF-β1, IL-5, IFN-γ, and IL-4 were detected by ELISA. We showed that TGF-β1, IL-5, and IL-4 levels in the TAC group were lower than the control group ($p<0.05$). Importantly, treatment with TAC+VD substantially reduced TGF-β1, IL-5, and IL-4 levels and those levels appeared similar to their normal conditions. No difference was seen between the TAC+VD group and the control group. Similarly, while the IFN-γ level in the model group and the TAC group was lower than that in the normal group ($p<0.05$), TAC+VD treatment brought those level back to normal (Figure 4).

TAC+VD regulates the NF-κB/TLR4 pathway

Given that TAC+VD treatment potently reduced immunological response in IgAN rats, we reasoned that the NF-κB/TLR4 pathway, which plays an important role in immunological response, was affected by TAC+VD treatment. The protein expression levels of p-P65, P65, and TLR4 were detected by western blot. We showed that p-P65/P65 and TLR4 levels were significantly downregulated by TAC and TAC+VD treatment. Particularly, p-P65/P65 and TLR4 were even lower in the TAC+VD group compared to the TAC group ($p<0.05$). No significant difference in p-P65/P65 and TLR4 levels was seen between the TAC+VD group and the normal group ($p>0.05$) (Figure 5).

Discussion

Here we show that TAC+VD therapy demonstrated superior protection compared to TAC single therapy against renal

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

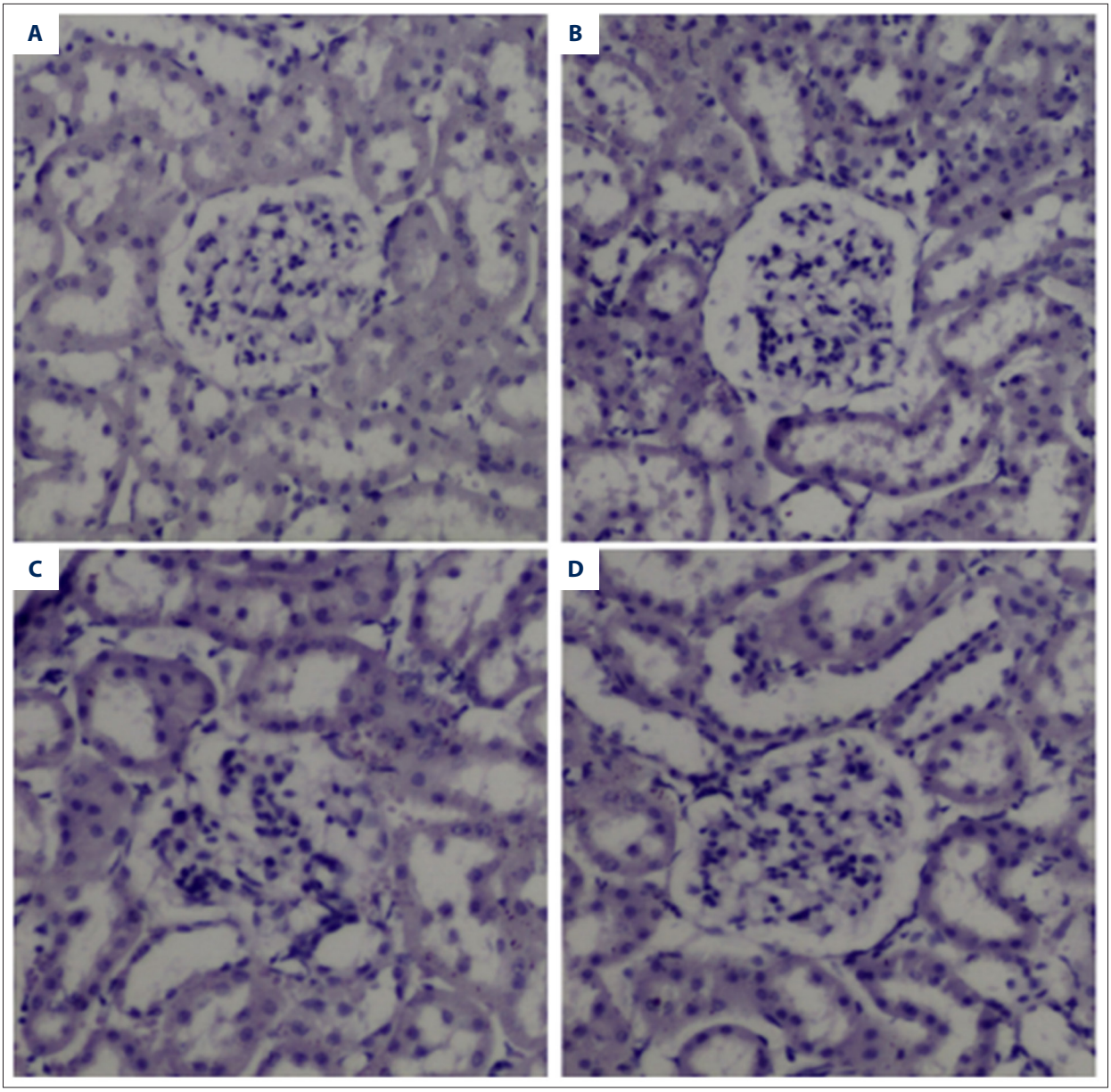

Figure 2. H&E staining of renal tissue (100×) harvested from (**A**) the control group; (**B**) the model group, (**C**) the TAC group, and (**D**) the TAC+VD group.

tissue damage in IgAN rats. We constructed a rat IgAN rat model through BSA and LPS immunization. This method has been used in a number of studies and the IgAN rat model has demonstrated substantial renal damages, as manifested by abnormal serum and urine biochemical parameters, which mimic clinical conditions [10–12]. In accordance with these findings, we showed that these rats demonstrated proteinuria and dysregulated protein levels in serum, as exemplified by elevated levels of Scr, BUN, and UAE and decreased level of ALB due to impaired kidney function. Histological analysis also corroborated disrupted renal tissue physiology, infiltration of inflammatory cells, and hyperplasia. Based on our results, at the time of treatment, IgAN rats had already developed late-stage IgAN. As IgAN is an immune-complex-mediated glomerulonephritis, immunosuppressants are commonly used for the treatment of the disease. TAC treatment can significantly reduce the dysregulation of Scr, BUN, ALB, and UAE levels [13,14]; studies also found that both TAC and vitamin D have good effect with regards to kidney injury recovery [15,16]. In our study, TAC+VD therapy demonstrated higher potency in normalizing these levels. These protective effects of TAC and TAC+VD therapy were also confirmed by histological analysis. Importantly, vitamin D enhanced suppression of immunological response in IgAN rats, as evidenced by lower

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

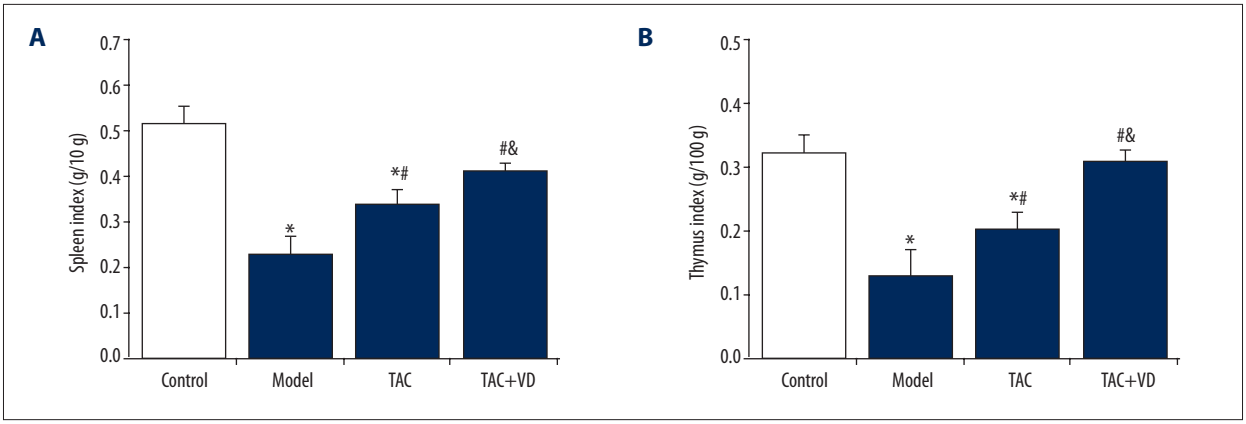

Figure 3. Changes in rats of (**A**) thymus index and (**B**) spleen index; * $p<0.05$ compared with the control group; # $p<0.05$ compared with the model group; & $p<0.05$ compared with the TAC group.

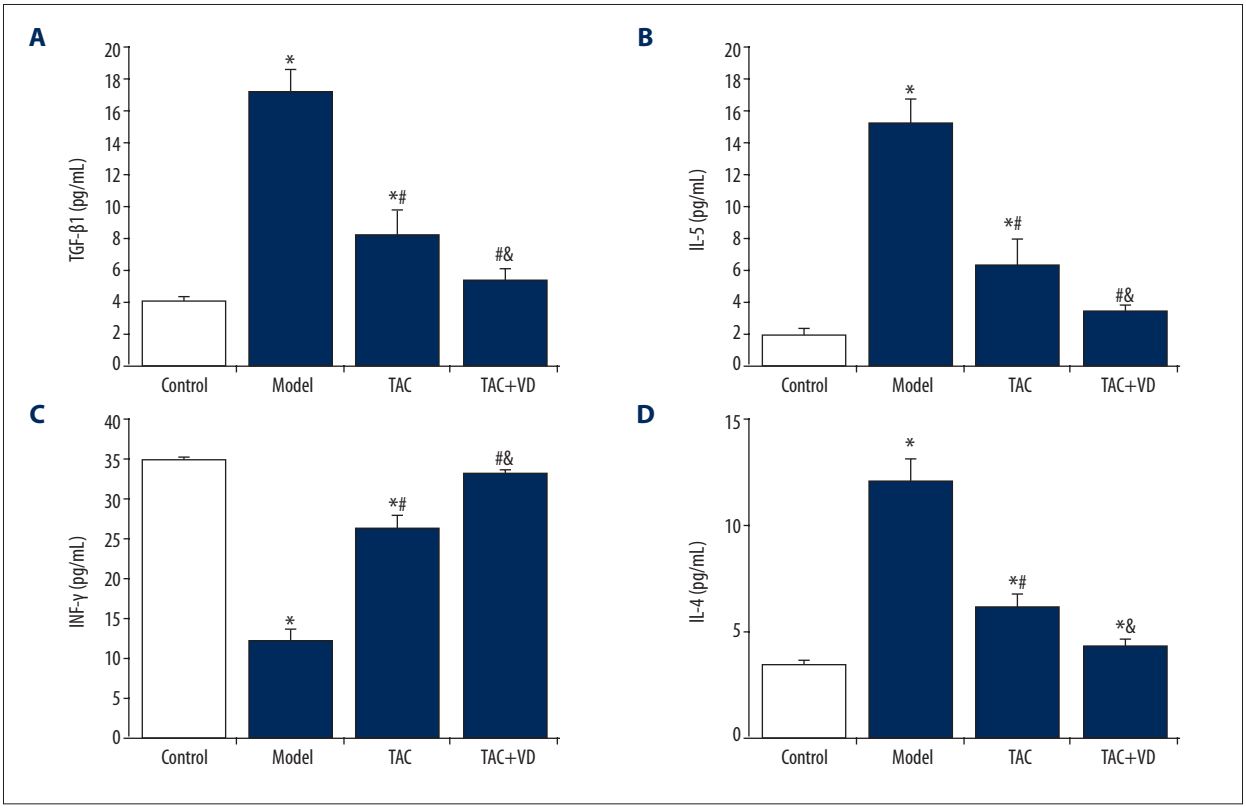

Figure 4. The levels in rats of (**A**) TGF-β1, (**B**) IL-5, (**C**) IFN-γ, and (**D**) IL-4; *$p < 0.05$ compared with the normal group; # $p<0.05$ compared with the model group; & $p<0.05$ compared with the TAC group.

levels of TGFβ1, IL-5, and IL-12. The effect of TAC in promoting T-cell activation was also augmented by vitamin D administration based on higher IFN-γ levels. Conventionally, vitamin D is thought to contribute to renal protection by modulating calcium metabolism and vascular function. Our data indicated that vitamin D also aids in immunosuppression. In line with this, in our study, less infiltration of inflammatory cells was seen in TAC+VD therapy compared to TAC single therapy. Recently, the role of vitamin D in regulating immune system has been implicated [17,18]. For example, in a recent study, vitamin insufficiency has been associated with an increased risk of renal transplantation failure [17]. Efforts have also been made in demonstrating the benefit of vitamin D supplementation after organ transplantation [17]. Our study, for the first time, potentiates vitamin D supplementation as a way to improve the immunosuppression in IgAN.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

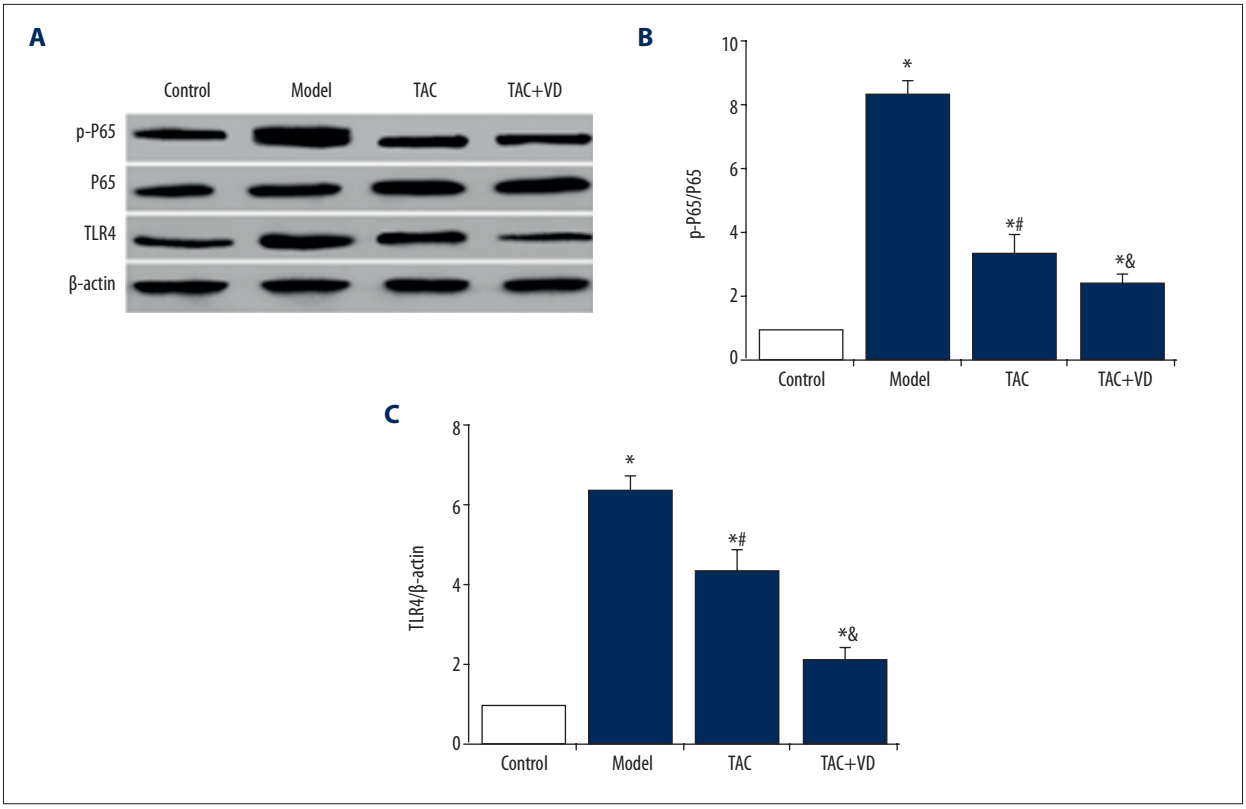

Figure 5. Western blot detection of NF-κB/TLR4-related protein expression levels: (**A**) protein map, (**B**) p-P65/P65 expression, (**C**) TLR4 expression; * $p<0.05$ compared with the normal group; # $p<0.05$ compared with the model group; & $p<0.05$ compared with the TAC group.

To further elucidate the mechanism of improved efficacy of TAC+VD therapy, we examined how the NF-κB/TLR4 pathway was affected by this treatment. The NF-κB/TLR4 pathway is well-known for regulating immunological response [19]. Evidence has indicated that the NF-κB/TLR4 pathway activity is markedly increased in IgAN patients, and that the level of the NF-κB/TLR4 pathway is associated with poorer outcomes of IgAN patients [20,21]. As an immunosuppressant, TAC downregulates the NF-κB/TLR4 pathway in a protection effect in transplantation and IgAN patients [22–24]. In our study, we showed that the ratio of p-P65/P65 was higher in IgAN rats, which was downregulated by TAC. Similarly, TLR4 was also downregulated by TAC. However, the limited immunosuppressive capacity of TAC demands that more potent immunosuppressive therapy be devised. In our study, TAC+VD therapy was able to enhance downregulation of TLR4 and p-P65/P65, which is in agreement to our hypothesis that vitamin D also served as an immunosuppressant in IgAN rats. This result is consistent with previous findings that vitamin D is able to downregulate the NF-κB/TLR4 pathway in a wide range of diseases [24]. Vitamin D is therefore frequently used in combination of other immunoregulators to achieve better therapeutic outcome [9,24,25]. For instance, vitamins have been tested in combination with progesterone in patients with traumatic brain injury, whereby an enhanced modulation of inflammatory response was seen. Taken together, the vascular stabilization and immunosuppressive role of vitamin D qualify it as a valuable supplement in the treatment of IgAN. Our study was similar to a number of studies that combined nutrient supplements in therapy of IgAN [26]; their non-toxicity as well as broad-spectrum benefit makes them desirable regimens in alleviating chronic diseases like IgAN.

Conclusions

Our study provided evidence that vitamin D is potent in augmenting renal function protection in IgAN rats. We suggest that TAC and vitamin D combinatorial therapy can be used in other diseases characterized by abnormal immunological and inflammatory activity. This novel treatment strategy is worthy of being tested in the clinical arena and could possibly improve the therapeutic outcome and quality of life in IgAN patients.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

References:

1. Wyatt RJ, Julian BA: IgA nephropathy. New Engl J Med, 2013; 368: 2402–14
2. Roberts IS: Pathology of IgA nephropathy. Nat Rev Nephrol, 2014; 10: 445–54
3. Zhang Q, Shi S-f, Zhu L et al: Tacrolimus improves the proteinuria remission in patients with refractory IgA nephropathy. Am J Nephrol, 2012; 35: 312–20
4. Li CJ, Li L: Tacrolimus in preventing transplant rejection in Chinese patients – optimizing use. Drug Des Devel Ther, 2015; 9: 473–85
5. Ballarin J, Poveda R, Ara J et al: Treatment of idiopathic membranous nephropathy with the combination of steroids, tacrolimus and mycophenolate mofetil: Results of a pilot study. Nephrol Dial Transplant, 2007; 22: 3196–201
6. Wan QJ, Hu HF, He YC et al: Tacrolimus combined with low-dose corticosteroids is an effective and safe therapeutic option for refractory IgA nephropathy. Exp Ther Med, 2016; 12: 1934–38
7. Li XH, Huang XP, Pan L et al: Vitamin D deficiency may predict a poorer outcome of IgA nephropathy. BMC Nephrol, 2016; 17: 164
8. Mann MC, Hemmelgarn BR, Exner DV et al: Vitamin D supplementation is associated with stabilization of cardiac autonomic tone in IgA nephropathy. Hypertension, 2015; 66: E4–E6
9. Jiang J, Shi D, Zhou XQ et al: Vitamin D inhibits lipopolysaccharide-induced inflammatory response potentially through the Toll-like receptor 4 signalling pathway in the intestine and enterocytes of juvenile *Jian carp* (*Cyprinus carpio var. Jian*). Br J Nutr, 2015; 114: 1560–68
10. Tian JH, Wang YH, Guo HX, Li RS: The Akt/mTOR/p70S6K pathway is activated in IgA nephropathy and rapamycin may represent a viable treatment option. Exp Mol Pathol, 2015; 99: 435–40
11. Tian J, Wang Y, Zhou X et al: Rapamycin slows IgA nephropathy progression in the rat. Am J Nephrol, 2014; 39: 218–29
12. Katzir Z, Leibovitch E, Vaknin H et al: Effect of atorvastatin on IgA nephropathy in the rat. Clin Nephrol, 2013; 79: 214–20
13. Wen Y, Liu L, Zhou P et al: Tacrolimus restores podocyte injury and stabilizes the expression of Cabin1 in 5/6 nephrectomized rats. Ren Fail, 2016; 38: 564–70
14. Liao R, Liu Q, Zheng Z et al: Tacrolimus protects podocytes from injury in lupus nephritis partly by stabilizing the cytoskeleton and inhibiting podocyte apoptosis. PLoS One, 2015; 10: e0132724
15. Okada S, Shikata K: A commentary on the VITAL study: Does vitamin D (receptor activation) protect againstnephropathy in type 2 diabetes? J Diabetes Investig, 2012; 3: 36–38
16. Kim YC, Chin HJ, Koo HS, Kim S: Tacrolimus decreases albuminuria in patients with IgA nephropathy and normal blood pressure: A double-blind randomized controlled trial of efficacy of tacrolimus on IgA nephropathy. PLoS One, 2013; 8: e71545
17. Hesketh CC, Knoll GA, Molnar AO et al: Vitamin D and kidney transplant outcomes: A protocol for a systematic review and meta-analysis. Syst Rev, 2014; 3: 64
18. Pludowski P, Holick MF, Pilz S et al: Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality – A review of recent evidence. Autoimmun Rev, 2013; 12: 976–89
19. Baeuerle PA, Henkel T: Function and activation of NF-kappaB in the immune system. Ann Rev Immunol, 1994; 12: 141–79
20. Liang S, Cai GY, Duan ZY et al: Urinary sediment miRNAs reflect tubulointerstitial damage and therapeutic response in IgA nephropathy. BMC Nephrol, 2017; 18: 63
21. Silva GE, Costa RS, Ravinal RC et al: NF-κB expression in IgA nephropathy outcome. Dis Markers, 2011; 31: 9–15
22. Nakamura H, Aoki M, Tamai K et al: Prevention and regression of atopic dermatitis by ointment containing NF-κB decoy oligodeoxynucleotides in NC/Nga atopic mouse model. Gene Ther, 2002; 9: 1221–29
23. Tanaka T, Fujita M, Bottino R et al: Endoscopic biopsy of islet transplants in the gastric submucosal space provides evidence of islet graft rejection in diabetic pigs. Islets, 2016; 8: 1–12
24. Tang H, Hua F, Wang J et al: Progesterone and vitamin D combination therapy modulates inflammatory response after traumatic brain injury. Brain Inj, 2015; 29: 1–10
25. Klampfer L: Vitamin D and colon cancer. World J Gastrointest Oncol, 2014; 6: 430–37
26. Hu J, Liu Z, Zhang H: Omega-3 fatty acid supplementation as an adjunctive therapy in the treatment of chronic kidney disease: A meta-analysis. Clinics, 2017; 72: 58–64

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]