International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2023.305.312

Research Article
Synovial Knee Joint in Rheumatoid Arthritis Treated with TDZD-8: An Association between Synovial Fibrosis and the TNF-α/VEGF/α-SMA Axis

[1]Saeed M. Alqahtani, [2]Zenat Ahmed Khired, [3]Amal Fahmy Dawood, [4]Mohammad Y. Alshahrani, [5]Norah M. Alzamil, [6]Bahjat S. Al-Ani and [6]Fahaid H. Al-Hashem

[1]Department of Orthopedic Surgery, College of Medicine, King Khalid University, Abha 62529, Saudi Arabia
[2]Department of Surgery, Jazan University, Jazan, Saudi Arabia
[3]Department of Basic Medical Sciences, College of Medicine, Princess Nourah bint Abdulrahman University, P.O. Box. 84428, Riyadh 11671, Saudi Arabia
[4]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Khalid University, Abha 61413, Saudi Arabia
[5]Department of Clinical Science, Family Medicine, College of Medicine, Princess Nourah bint Abdulrahman University, P.O. Box. 84428, Riyadh 11671, Saudi Arabia
[6]Department of Physiology, College of Medicine, King Khalid University, Abha 62529, Saudi Arabia

Abstract

Background and Objective: Inflammation and fibrosis-induced swelling are key features in rheumatoid arthritis (RA) that causes joint damage and disability. The inflammatory biomarker, Tumor Necrosis Factor-Alpha (TNF-α) is known to be involved in RA pathology. The RA can cause synovial membrane fibrosis through the activation of the TNF-α/Vascular Endothelial Growth Factor (VEGF)/α-smooth muscle actin (α-SMA) pathway, which can be inhibited by TDZD-8, the inhibitor of the enzyme Glycogen Synthase Kinase-3β (GSK3β). **Materials and Methods:** The RA was induced in rats using active immunization by injection with collagen type II (COII). A matched group of rats was treated with 1 mg kg^{-1} TDZD-8 (COII+TDZD-8) for 3 weeks after the immunization protocol. At the end of the experiment (week 6), blood and synovium tissue samples were collected. **Results:** The development of RA was established and confirmed by a sharp increase in the blood levels of the rheumatoid factor (RF) as well as swelling and deformities in the hind limbs. Blood levels of TNF-α and synovium tissue levels of VEGF, α-SMA and collagen fibers were significantly ($p<0.0001$) increased in the model group (RA). The TDZD-8 was significantly associated with the inhibition of all these parameters and curing the swelling and deformities in the hind limbs. In addition, a significant correlation between RF, TNF-α, VEGF, α-SMA and synovial fibrosis was observed. **Conclusion:** These findings in a rat model of rheumatoid arthritis that affects synovial knee joint demonstrate an association between the fibrosis in the synovial membrane and the synovium TNF-α/VEGF/α-SMA axis while being treated by TDZD-8.

Key words: Rheumatoid arthritis, knee joint, TNF-α/VEGF/α-SMA axis, fibrosis, TDZD-8

Citation: Alqahtani, S.M., Z.A. Khired, A.F. Dawood, M.Y. Alshahrani, N.M. Alzamil, B.S. Al-Ani and F.H. Al-Hashem, 2023. Synovial knee joint in rheumatoid arthritis treated with TDZD-8: An association between synovial fibrosis and the TNF-α/VEGF/α-SMA axis. Int. J. Pharmacol., 19: 305-312.

Corresponding Author: Fahaid H. Al-Hashem, Department of Physiology, College of Medicine, King Khalid University, Abha 61421, Saudi Arabia
Tel: 966-500142929

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rheumatoid arthritis is an autoimmune chronic disease that affects women more than men and is characterised by inflammation, swelling and deformities of joints[1,2]. This disease (RA) that affects 1% of the population in the world cannot be treated, but it can be manged by medications[3]. People with RA have increased risk of other diseases such as cardiovascular and lung diseases due to systemic inflammation that extends beyond the knee joint[4,5]. The TNF-α as well as the key regulator of new blood vessel formation from existing blood vessels (angiogenesis), VEGF is associated with RA pathology[6]. The synovium is a notable part of the joint's structure, which is injured in rheumatic diseases[7]. The VEGF interacts with synoviocytes and increases bone resorption via augmentation of osteoclasts activities, which is one of the hallmark characteristics of RA[7]. In addition, several growth and transcription factors such as Transforming Growth Factor-β (TGF-β) and NF-κB as well as cytokines like Interleukin-6 (IL-6), Interleukin-1 (IL-1) and TNF-α contribute to the VEGF regulation[6]. The TNF-α mediated the nuclear translocation of NF-κB leading to the expression of the proinflammatory cytokine gene in synoviocytes as well as matrix metalloproteinase production and the destruction of cartilage in the joint[8]. Furthermore, collagen expression in cardiac fibroblasts is dependent on the profibrogenic biomarker α-SMA that participates in myocardial fibrosis[9] and synovium cells expression of α-SMA[10].

The enzyme GSK3β has pleiotropic effects in diverse systems (I) Participated in the pathophysiology of rheumatic diseases since inhibiting GSK-3β reduced collagen-induced RA, cartilage matrix degradation in a cultured chondrogenic cell line and increased in a mouse model of osteoarthritis the thickness of bones[11], (II) Played a role in neuroinflammation and pain[12], (III) proposed as a potential link between diabetes and Alzheimer disease[13] and (IV) Inhibited IL-10, the anti-inflammatory cytokine[14]. Thus, this study explored in a rat model of RA, the synovium TNF-α/VEGF/α-SMA axis mediated fibrosis in the presence and absence of the specific pharmacological inhibitor of GSK3β, TDZD-8.

MATERIALS AND METHODS

Study area: This study was conducted at the Research Centers, College of Medicine, Princess Nourah University and King Khalid University, Saudi Arabia from February, 2022 to June, 2022.

Animals: Wistar rats (160±10 g) were used to carry this work endorsed by the Princess Nourah University (Ethical Committee, IBR No. 17-0201). Animals were kept in clean cages inside the animal house provided with a cycle of 12 hrs light/dark and a constant room temperature as well as having free access to water and food.

Experimental design: After the acclimatization period, rats were separated equally into three groups (n = 8 per group). The model group (RA): Rats were immunized (active immunization) with collagen type II (COII) (bovine source, Sigma-Aldrich, MO, USA) as reported before[15], which was confirmed after 21 days. The treated group (COII+TDZD-8): matched immunized rats started the treatment with 1 mg kg^{-1} TDZD-8 at day 21 for three more weeks[16]. The control group: Untreated rats received vehicles via the same route, intraperitoneal injections. Blood was withdrawn under anesthesia before sacrificing the animals. Synovia were collected, snap-frozen in liquid nitrogen and stored until being used at -80°C.

Histological examination: Synovium tissue samples were immediately fixed for about 15 hrs in 10% formalin. Paraffin blocks were prepared and sections (5 μm thick) were deparaffinized and processed for Masson's trichrome staining as previously described by Dawood *et al.*[17]. This stain detects collagen fibers deposited in synovium that reflects the degree of fibrosis.

VEGF and α-SMA immunohistochemistry: Synovium tissue sections (5 μm thick) obtained from all rats groups were deparaffinized and processed as described before by Dawood *et al.*[17]. Rabbit Anti-Human VEGF-A polyclonal antibody (Catalog No. SPC-1291, StressMarq Biosciences, Victoria, BC, Canada) that has species reactivity with human, mouse and rat and anti-α-SMA obtained from abcam, Cambridge, UK were used at a dilution 1/200 as the primary antibody added to tissue sections in a humidity chamber for 60 min at room temperature. The secondary antibody was added after washing the slides and left for half an hour at room temperature. Sections were then counterstained with Meyer hematoxylin. The areas percentage of VEGF and α-SMA immunostaining and collagen fibers deposition was evaluated using the "Leica Qwin 500 C" image analyzer (Cambridge, UK). The ANOVA followed by *post hoc* analysis (Tukey's Test) was used for comparing the quantitative data, which is presented as Mean±Standard Deviations (SD). The $p<0.05$ was deemed statistically significant.

Rheumatoid factor (RF) and TNF-α enzyme-linked immunosorbent assay: The ELISA kits for blood determination of RF (CUSABIO Technology LLC, TX, USA) and TNF-α (Abcam, Cambridge, UK) in all rats' group at the end of the investigation at week 6 were used as suggested by the manufacturer.

Statistical analysis: Statistical software package (GraphPad Prism, version 6) was used to carry out the statistical analysis. In order to calculate the differences among the involved groups of rats, one-way ANOVA was performed followed by Tukey's Test. Data were expressed as Mean±SD and results were considered significant when $p \leq 0.05$.

RESULTS

TDZD-8 is associated with the inhibition of RF, TNF-α and VEGF augmented by COII immunization: The TNF-α is increased in the blood and synovial fluid obtained from children with chronic arthritis and VEGF upregulation is associated with rheumatic diseases and is located downstream of TNF-α. However, the effect of TDZD-8, the inhibitor of the enzyme GSK3β that is well-known to contribute to RA on the augmentation of TNF-α/VEGF axis in RA has not been investigated before. Therefore, blood levels of the RA biomarker (RF) and the inflammatory biomarker (TNF-α) and synovium tissue levels of VEGF have assessed in all rat groups 6 weeks post COII immunization in the presence and absence of TDZD-8. A sharp increase in RF (Fig. 1a) and TNF-α (Fig. 1b) as well as tissue protein expression of VEGF (Fig. 1c) that was significantly ($p<0.0001$) inhibited by TDZD-8 was observed. However, the level of RF and TNF-α in the animal group treated with TDZD-8 (COII+TDZD-8) was still significantly ($p<0.0001$) higher when compared with the control untreated rats, which pointed to a partial inhibition. Whereas, complete inhibition of VEGF tissue expression was achieved by TDZD-8 since its levels were comparable to the control group. Compared to weak VEGF-positive immunostained cells depicted in the control group (Fig. 1d), active immunization with COII caused strong widespread VEGF positive immunostained cells in all layers of the synovial tissue of the model group (RA) (Fig. 1e) that appeared to be substantially inhibited by TDZD-8 in the treated group (COII+TDZD-8).

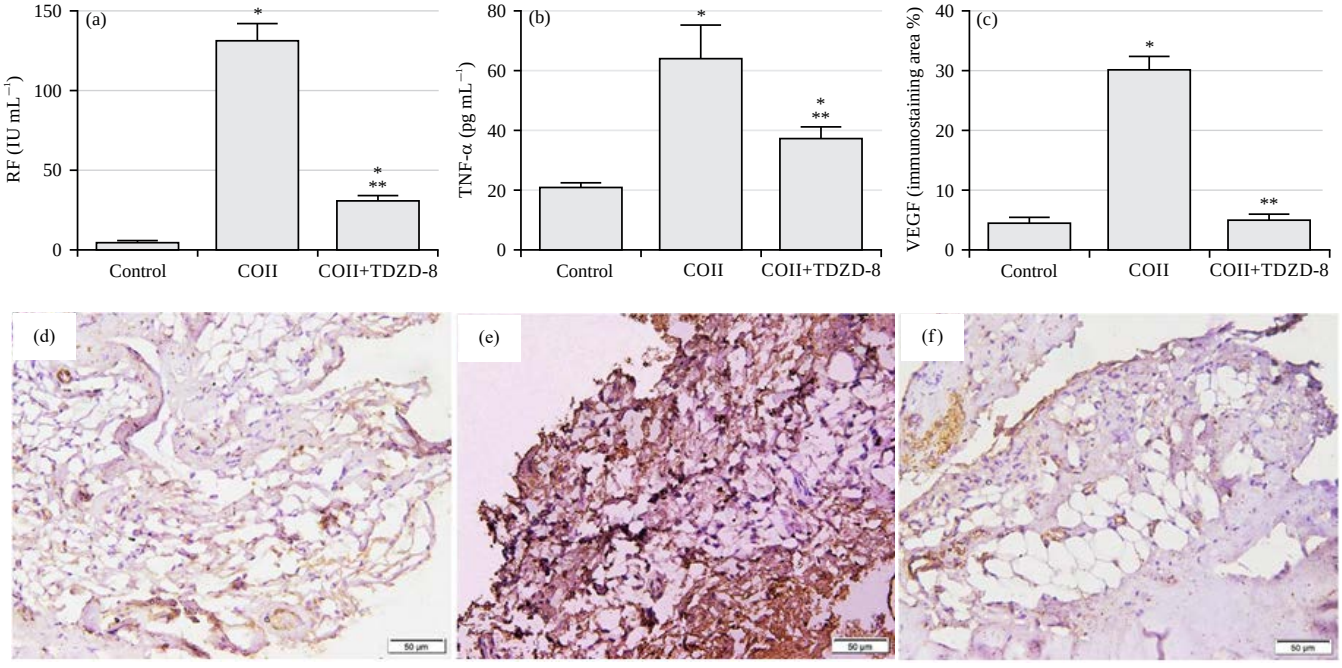

Fig. 1(a-f): COII immunization activates biomarker of RA and TNF-α/VEGF axis with inhibition being associated with TDZD-8. Blood levels of (a) RF and (b) TNF-α as well as (c-f) Synovium tissue levels of VEGF were measured end of week 6 in all groups of rats Control group, model group (COII) and treated group (COII+TDZD-8). The VEGF immunohistochemistry representative images (×200) of synovium sections prepared at the end of the experiment, end of week 6 from the (d) Control group, (e) Model group (COII) and (f) Treated group (COII+TDZD-8) are displayed

Presented p-values are significant. *p<0.0001 vs control, **p<0.0001 vs COII. RA: Rheumatoid arthritis, RF: Rheumatoid factor, TNF-α: Tumor Necrosis Factor-alpha, VEGF: Vascular Endothelial Growth Factor, COII: Collagen type II and TDZD-8: Thiadiazolidine derivative

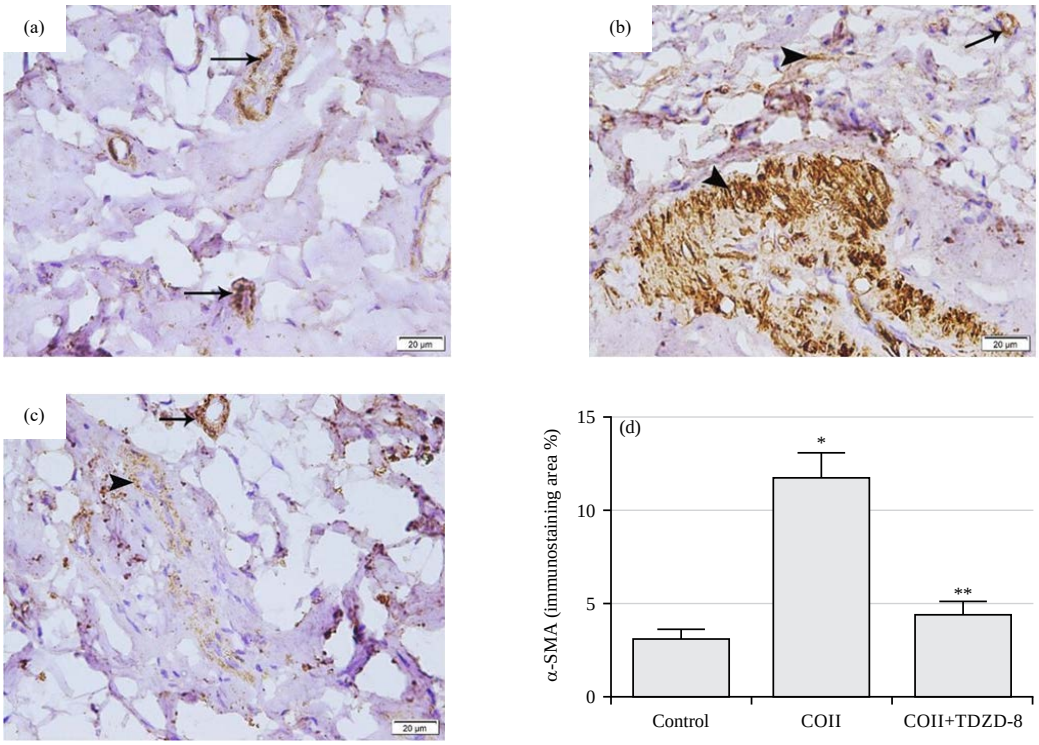

Fig. 2(a-d): COII immunization activates synovium α-SMA protein expression with inhibition being associated with TDZD-8. α-SMA immunohistochemistry representative images (×400) of synovium sections prepared end of week 6 from the (a) Control rats, (b) Model rats (COII) and (c) Treated rats (COII+TDZD-8) are displayed and (d) A quantitative analysis of α-SMA immunostaining deduced from these images

Presented p-values are significant, *p = 0.0002 vs control, ***p<0.0001 vs COII, α-SMA: Alpha-smooth muscle actin, COII: Collagen type II and TDZD-8: Thiadiazolidine derivative

TDZD-8 is associated with the inhibition of α-SMA augmented in injured synovium by COII immunization: In cell signalling, α-SMA is located downstream of VEGF. Therefore, in view of the upregulation of VEGF in our model of RA that was inhibited with TDZD-8, we assessed α-SMA protein expression in the synovial knee joint tissue in all rats groups. Compared to weak positive immunostaining cells in the blood vessels' smooth muscles (arrow) of the control group (Fig. 2a), immunohistochemical staining of α-SMA showed that RA increased the number and intensity of α-SMA positive immunostaining cells in the stroma of the synovial tissue (arrow head) and besides the wall of the blood vessels (arrow) (Fig. 2b). The TDZD-8 treatment for 3 weeks appeared to substantially decrease α-SMA positive immunostaining cells in the synovial tissue (arrow head) and besides the wall of the blood vessels (arrow) (Fig. 2c-d), but still significant in comparison with the control rats.

TDZD-8 is associated with the inhibition of fibrosis augmented in injured synovium by COII immunization: The VEGF and α-SMA promotes synovial fibrosis in osteoarthritis. To evaluate the association of TNF-α/VEGF/α-SMA axis with synovium fibrosis, synovial fibrosis was assessed with and without TDZD-8 incorporation. Masson trichrome-stained sections of the control group (Fig. 3a) showed weak positive staining, compared to a coarse collagen deposition in all layers of the synovium prepared from the model group (COII) (Fig. 3b). The TDZD-8 treatment substantially but not completely inhibited the deposition of collagen since moderate collagen deposition in the subintimal region and scattered between adipocytes are observed (Fig. 3c-d).

Correlation between synovial fibrosis score and synovial TNF-α/NF-κB/VEGF/α-SMA axis as well as biomarker of RA: The correlation between the score of collagen deposition (fibrosis) in synovium tissue and the investigated axis was determined to link these parameters in a rat model of RA. Knee joint synovium fibrosis score demonstrated a significant ($p<0.0001$) positive correlation with TNF-α ($r = 0.992$) (Fig. 4a), VEGF ($r = 0.979$) (Fig. 4b), α-SMA ($r = 0.964$) (Fig. 4c) and RF ($r = 0.985$) (Fig. 4d).

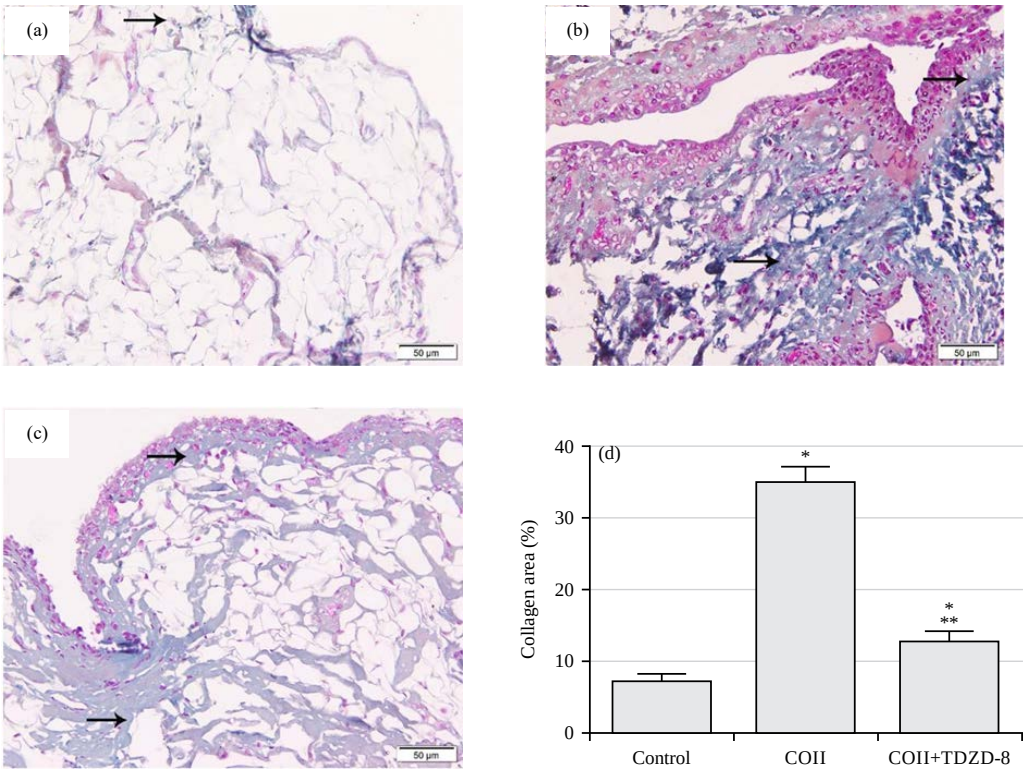

Fig. 3(a-d): TDZD-8 inhibits COII immunization-induced synovial fibrosis in rats, (a-c) Masson's trichrome stained images (×200) for synovium sections of (a) Control group, (b) COII group and (c) COII+TDZD-8 group are displayed. Arrows point to collagen fibers deposition. Histograms in (d) show a quantitative analysis of fibrosis (collagen area (%)) in synovium sections from the above groups

COII: Collagen type II and TDZD-8: Thiadiazolidine derivative

Based on the above data, a proposed model of rheumatoid arthritis that link the inflammation, angiogenesis and fibrosis in the pathogenesis of rheumatoid arthritis which appeared to be inhibited by TDZD-8 was shown in (Fig. 5).

DISCUSSION

Using a rat model of knee joint RA induced by active immunization with bovine collagen type II, this report examined the working hypothesis that pointed to the potential activation of TNF-α/VEGF/α-SMA axis-mediated synovium fibrosis as well as the potential inhibition of this axis with the pharmacological inhibitor of the enzyme GSK3β, TDZD-8. Also, a link between these parameters and synovium fibrosis was investigated. The data presented here confirmed the induction of RA in these rats as demonstrated by a sharp increase in the blood levels of RF and TNF-α, besides changes in the macroscopic features of paws in rats, which was associated with the augmentation of VEGF and α-SMA protein expression in the synovium of the knee joint, as well as a sharp increase in the formation of collagen fibers in the synovium, which were effectively inhibited by TDZD-8 (Fig. 5). Moreover, a significant positive correlation between TNF-α/VEGF/α-SMA axis and knee joint fibrosis (Fig. 4) was additional support to the above stated working hypothesis.

The damage to the joints caused by RA negatively affects the quality of life of the person carrying the disease since there is no cure for RA, leaving the management of the disease as the only available option. Therefore, investment in the discovery of a new signalling pathway associated with RA is needed to further understand the pathophysiology of the disease that may help to cure RA in the future. Thus, the signalling pathway investigated in this work (TNF-α/VEGF/α-SMA axis- mediated fibrosis) with and without the incorporation of TDZD-8 is a new approach through the involvement of these parameters is known to participate in rheumatic diseases. The TNF-α inhibitor, infliximab decreased VEGF levels in patients with RA[18] and TNF-α augmented the production of VEGF by chondrocytes harvested from osteoarthritis patients[19], which demonstrated the pathological

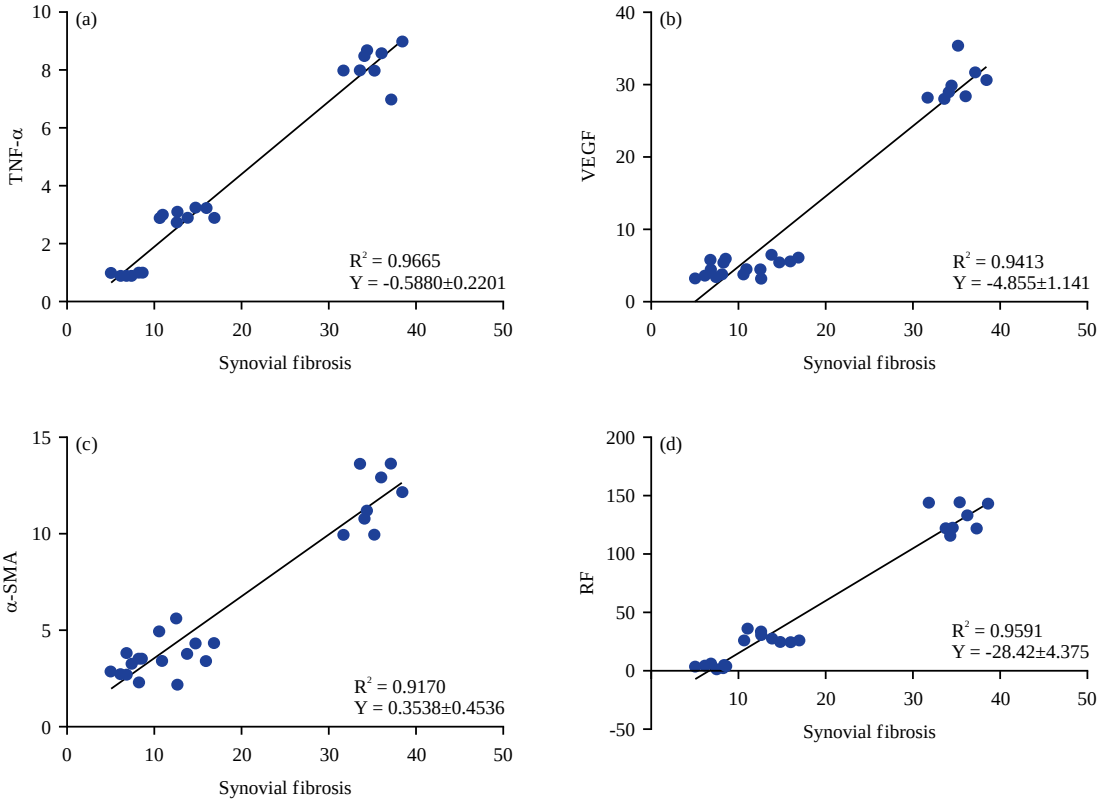

Fig. 4(a-d): Correlation between the scoring of fibrosis collagen fibers deposition and TNF-α/VEGF/α-SMA axis-mediated fibrosis and arthritis. Degree of the deposition of collagen fibers in synovium was evaluated in all rats' group end of week 6 to link between fibrosis and (a) TNF-α, (b) VEGF, (c) α-SMA and (d) RF

TNF-α: Tumor Necrosis Factor-alpha, VEGF: Vascular Endothelial Growth Factor, α-SMA: Alpha-smooth muscle actin and RF: Rheumatoid factor

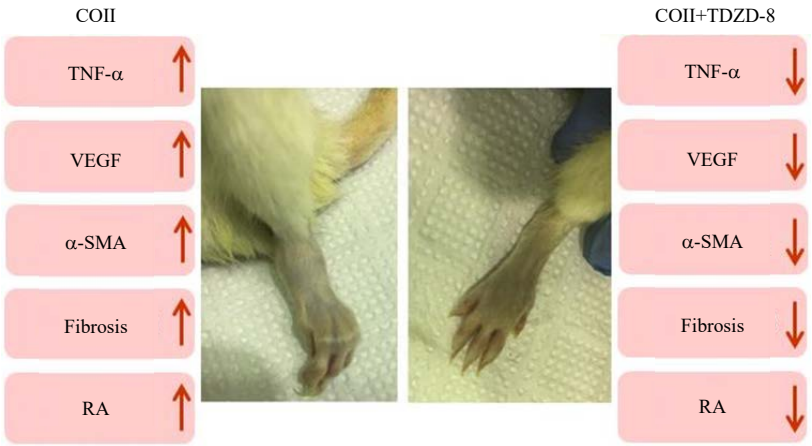

Fig. 5: Proposed model for rheumatoid arthritis TDZD-8

COII: Collagen type II, TDZD-8: Thiadiazolidine derivative, TNF-α: Tumor Necrosis Factor-alpha, VEGF: Vascular Endothelial Growth Factor, α-SMA: Alpha-smooth muscle actin and RA: Rheumatoid arthritis

role of these molecules in diseases affecting the joints, as well as placing TNF-α upstream of VEGF in cell signalling. The increase in the expression of VEGF in rheumatic diseases was reported, (I) In RA pathology since it causes synovial angiogenesis, a process that leads to RA[6]. In addition, expression of VEGF is increased in synovial macrophages and fibroblasts of patients with RA[6], (II) In synovia obtained from patients with osteoarthritis that was positively

correlated with the severity of the disease[20], (III) In blood obtained from Systemic Lupus Erythematosus (SLE) patients that demonstrated a significant correlation between VEGF levels and SLE disease activities[21] and (IV) In juvenile idiopathic arthritis that demonstrated a significant correlation between VEGF serum levels and disease activities as well as the augmentation of IL-1, IL-6 and TNF-α[22] and (V) in ankylosing spondylitis, which is treated with the TNF-α inhibitors[6]. All these reports together with the study that demonstrated the upregulation of the inflammatory cell infiltration like macrophages, leukocytes and lymphocytes as well as α-SMA expression in the synovium of the hip joint of sever developmental dysplasia of the hip[23], were in agreement with the findings presented in this study that points to the upregulation of synovial TNF-α, VEGF and α-SMA in knee joint RA (Fig. 1-2). In addition, synovial tissue inflammation, TNF-α, Nuclear Factor Kappa B (NF-κB), VEGF and α-SMA promoted synovial fibrosis in osteoarthritis[24,25] and *in vitro* study demonstrated that VEGF was able to directly induce the synthesis of collagen in dermal fibroblasts[26], which were also in parallel with the data shown in Fig. 3.

The TDZD-8 was reported to inhibit the enzyme GSK3β in different systems. For example, (I) TDZD-8 maintained water homeostasis in a rat model of acute kidney injury induced by renal ischemia reperfusion (I/R) injury by inhibiting the decrease in the number of a water channel (aquaporin-1) and sodium transporters caused by I/R injury[27], (II) TDZD-8 protected against amyloid-like aggregation that has a possible role in the pathogenesis of Alzheimer's disease[28], (III) GSK3β inhibitors attenuated pulmonary TNF-α, NF-κB and fibrosis[29,30], and (IV) TDZD-8 inhibited synovial leukocyte infiltration in RA rat model[15]. These studies are in parallel with the data shown in Fig. 1-4.

This study demonstrated that this animal model of rheumatoid arthritis may be useful in dissecting mechanisms of synovial knee joint injury. However, a fourth group of immunised rats treated with the drug of choice for rheumatoid arthritis, the immunosuppressive drug methotrexate would provide more strength to this work.

CONCLUSION

The data obtained from the synovium of knee joints in a rat model of rheumatoid arthritis induced by injecting bovine collagen type II into rats (active immunization approach) point to the critical role of the TNF-α/VEGF/α-SMA axis-mediated fibrosis in the pathogenesis of the inflammatory autoimmune disease rheumatoid arthritis, which appears to be inhibited by the treatment with TDZD-8 for a period of 21 days.

SIGNIFICANCE STATEMENT

This work represents an essential contribution to the area of rheumatic diseases that involve rheumatoid arthritis (RA) characterised by inflammation of joints which compromises the quality of life if not managed properly. Using a rat model of the disease generated by the active immunization method over a period of 3 weeks, blood levels of rheumatoid factor and TNF-α and synovial membrane protein expression of VEGF, α-SMA and collagen fibers were documented, which appeared to be inhibited by the pharmacological inhibitor of the enzyme GSK3β, TDZD-8. Further work will determine whether the treatment effect of TDZD-8 can be extended over a longer period of time.

ACKNOWLEDGMENTS

This work was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2023R110), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. This research was also funded by the Research Deanship of King Khalid University, Abha, Saudi Arabia, Grant No. RGP2/225/44. In addition, we are grateful to Dr. Mariam Al-Ani from Face Studio Clinic, 90 Hagley Road, Edgbaston, Birmingham, B16 8LU, UK for proofreading the manuscript.

REFERENCES

1. Ahlmen, M., B. Svensson, K. Albertsson, K. Forslind and I. Hafström, 2009. Influence of gender on assessments of disease activity and function in early rheumatoid arthritis in relation to radiographic joint damage. Ann. Rheumatic Dis., 69: 230-233.
2. Smolen, J.S., D. Aletaha, A. Barton, G.R. Burmester and P. Emery *et al*., 2018. Rheumatoid arthritis. Nat. Rev. Dis Primers, Vol. 4. 10.1038/nrdp.2018.1.
3. Chen, Z., D. Andreev, K. Oeser, B. Krljanac and A. Hueber *et al*., 2016. Th2 and eosinophil responses suppress inflammatory arthritis. Nat. Commun., Vol. 7. 10.1038/ncomms11596.
4. Scott, D.L., F. Wolfe and T.W.J. Huizinga, 2010. Rheumatoid arthritis. Lancet, 376: 1094-1108.
5. Bordy, R., P. Totoson, C. Prati, C. Marie, D. Wendling and C. Demougeot, 2018. Microvascular endothelial dysfunction in rheumatoid arthritis. Nat. Rev. Rheumatol., 14: 404-420.
6. van Le, T.H. and S.M. Kwon, 2021. Vascular endothelial growth factor biology and its potential as a therapeutic target in rheumatic diseases. Int. J. Mol. Sci., Vol. 22. 10.3390/ijms22105387.

7. Kim, W.U., S.S. Kang, S.A. Yoo, K.H. Hong and D.G. Bae *et al*., 2006. Interaction of vascular endothelial growth factor 165 with neuropilin-1 protects rheumatoid synoviocytes from apoptotic death by regulating Bcl-2 expression and Bax translocation. J. Immunol., 177: 5727-5735.
8. Tak, P.P. and G.S. Firestein, 2001. NF-κB: A key role in inflammatory diseases. J. Clin. Invest., 107: 7-11.
9. Harikrishnan, V., A.S. Titus, R.T. Cowling and S. Kailasam, 2019. Collagen receptor cross-talk determines α-smooth muscle actin-dependent collagen gene expression in angiotensin II-stimulated cardiac fibroblasts. J. Biol. Chem., 294: 19723-19739.
10. Vickers, S.M., L.L. Johnson, L.Q. Zou, I.V. Yannas, L.J. Gibson and M. Spector, 2004. Expression of α-smooth muscle actin by and contraction of cells derived from synovium. Tissue Eng., 10: 1214-1223.
11. Zahoor, M., P.H. Cha, D.S. Min and K.Y. Choi, 2014. Indirubin-3 -oxime reverses bone loss in ovariectomized and hindlimb-unloaded mice via activation of the Wnt/β-catenin signaling. J. Bone Miner. Res., 29: 1196-1205.
12. Maixner, D.W. and H.R. Weng, 2013. The role of glycogen synthase kinase 3 beta in neuroinflammation and pain. J. Pharm. Pharmacol., Vol. 1. 10.13188/2327-204X.1000001.
13. Zhang, Y., N.Q. Huang, F. Yan, H. Jin, S.Y. Zhou, J.S. Shi and F. Jin, 2018. Diabetes mellitus and Alzheimer's disease: GSK-3β as a potential link. Behav. Brain Res., 339: 57-65.
14. Chan, M.M.P., B.K.W. Cheung, J.C.B. Li, L.L.Y. Chan and A.S.Y. Lau, 2009. A role for glycogen synthase kinase-3 in antagonizing mycobacterial immune evasion by negatively regulating IL-10 induction. J. Leukocyte Biol., 86: 283-291.
15. Alzamil, N.M., F.A. Alradini, B. Al Ani, S. Younes, M.F. Saja, S.S. Kamar and A.F. Dawood, 2020. Inhibition of GSK3β protects against collagen type II induced arthritis associated with a decrease in synovial leukocyte infiltration and inhibition of endoplasmic reticulum stress and autophagy biomarkers. Clin. Exp. Pharm. Physiol., 47: 1393-1401.
16. Zhou, H., J. Liu, J. Zeng, B. Hu, X. Fang and L. Li, 2016. Inhibition of GSK-3β alleviates collagen II-induced rheumatoid arthritis in rats. Med. Sci. Monit., 22: 1047-1052.
17. Dawood, A.F., N.M. Alzamil, P.W. Hewett, M.A. Momenah and M. Dallak *et al*., 2022. Metformin protects against diabetic cardiomyopathy: An association between desmin-sarcomere injury and the iNOS/mTOR/TIMP-1 fibrosis axis. Biomedicines, Vol. 10. 10.3390/biomedicines10050984.
18. Strunk, J., E. Bundke and U. Lange, 2006. Anti-TNF-α antibody Infliximab and glucocorticoids reduce serum vascular endothelial growth factor levels in patients with rheumatoid arthritis: A pilot study. Rheumatol. Int., 26: 252-256.
19. Honorati, M.C., L. Cattini and A. Facchini, 2004. IL-17, IL-1β and TNF-α stimulate VEGF production by dedifferentiated chondrocytes. Osteoarthritis Cartilage, 12: 683-691.
20. Takano, S., K. Uchida, G. Inoue, T. Matsumoto and J. Aikawa *et al*., 2018. Vascular endothelial growth factor expression and their action in the synovial membranes of patients with painful knee osteoarthritis. BMC Musculoskelet Disord., Vol. 19. 10.1186/s12891-018-2127-2.
21. Barbulescu, A.L., A.F. Vreju, A.M. Buga, R.E. Sandu and C. Criveanu, 2015. Vascular endothelial growth factor in systemic lupus erythematosus-correlations with disease activity and nailfold capillaroscopy changes. Rom. J. Morphol. Embryol., 56: 1011-1016.
22. Świdrowska-Jaros, J. and E. Smolewska, 2018. A fresh look at angiogenesis in juvenile idiopathic arthritis. Central Eur. J. Immunol., 43: 325-330.
23. Li, D., H. Wang, J.Y. He, C.L. Wang and W.J. Feng *et al*., 2019. Inflammatory and fibrosis infiltration in synovium associated with the progression in developmental dysplasia of the hip. Mol. Med. Rep., 19: 2808-2816.
24. Zhang, L., R. Xing, Z. Huang, L. Ding and L. Zhang *et al*., 2021. Synovial fibrosis involvement in osteoarthritis. Front. Med., Vol. 8. 10.3389/fmed.2021.684389.
25. Benito, M.J., D.J. Veale, O. FitzGerald, W.B. van den Berg and B. Bresnihan, 2005. Synovial tissue inflammation in early and late osteoarthritis. Ann. Rheumatic Dis., 64: 1263-1267.
26. Maurer, B., A. Distler, Y.A. Suliman, R.E. Gay and B.A. Michel *et al*., 2013. Vascular endothelial growth factor aggravates fibrosis and vasculopathy in experimental models of systemic sclerosis. Ann. Rheumatic Dis., 73: 1880-1887.
27. Liu, Q., Y. Kong, X. Guo, B. Liang and H. Xie *et al*., 2021. GSK-3β inhibitor TDZD 8 prevents reduction of aquaporin-1 expression via activating autophagy under renal ischemia reperfusion injury. FASEB J., Vol. 35. 10.1096/fj.202100549R.
28. Balasubramaniam, M., N. Mainali, S.K. Bowroju, P. Atluri and N.R. Penthala *et al*., 2020. Structural modeling of GSK3β implicates the inactive (DFG-out) conformation as the target bound by TDZD analogs. Sci. Rep., Vol. 10. 10.1038/s41598-020-75020-w.
29. Jin, H., X. Yang, K. Zhao, L. Zhao, C. Chen and J. Yu, 2019. Glycogen synthase kinase-3 beta inhibitors protectagainst the acute lung injuries resulting from acute necrotizing pancreatitis. Acta Cirúrgica Bras., Vol. 34. 10.1590/s0102-865020190060000009.
30. Jeffers, A., W. Qin, S. Owens, K.B. Koenig and S. Komatsu *et al*., 2019. Glycogen synthase kinase-3β inhibition with 9-ING-41 attenuates the progression of pulmonary fibrosis. Sci. Rep., Vol. 9. 10.1038/s41598-019-55176-w.