Research Paper

Effects of glucocorticoids and tumor necrosis factor-alpha inhibitors on both clinical and molecular parameters in patients with Takayasu arteritis

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ABSTRACT

Objective: To explore the effect of sequential treatment with glucocorticoid and tumor necrosis factor-alpha inhibitors in patients with Takayasu arteritis (TA). **Materials and Methods:** In five patients with TA, the effects of the sequential treatment with prednisone for 5-7 months and then with adalimumab (ADA) + methotrexate (MTX) or infliximab + MTX, or with ADA only, for 12 months on both clinical and laboratory findings were evaluated. **Results:** All treatments improved both symptoms and laboratory parameters without the development of side-effects. **Conclusions:** It was hypothesized that MMP-9 and neutrophil gelatinase-associated lipocalin could be markers of the response to the treatments.

Key words: Adalimumab, infliximab, methotrexate, matrix metalloproteinases-9, neutrophil gelatinase-associated lipocalin, takayasu arteritis

INTRODUCTION

Takayasu arteritis (TA) is an idiopathic chronic inflammatory granulomatous disease affecting aorta and its main branches.[1,2] The pathogenesis of TA includes vessel injury due to inflammatory mediators, e.g., Tumor necrosis factor-α (TNF-α), released by T cells, natural killers, γ/δ T cells and macrophages;[3-5] However, an imbalance between matrix metalloproteinases (MMPs) and their tissue inhibitors of metalloproteinases (TIMPs) may also be involved.[6] In particular, it has been reported that MMPs 1, 3 and 9 were significantly higher in subjects with active disease.[7,8]

Previously, we documented an association between MMP-9 and neutrophil gelatinase-associated lipocalin (NGAL), a protein belonging to the lipocalin family. NGAL was able to modulate the activity of MMP-9 through the activation of the NGAL/MMP-9 complex.[9,10] NGAL plasma concentration has been associated with vascular diseases.[11]

The clinical manifestation of TA is related to both sites of affected vessel and severity of inflammation and the acute

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stage is represented by fever, weight loss, and elevated C-reactive protein (CRP) levels.[12] Unfortunately, due to the delay in diagnosing, patients experience claudication, absence of pulses, hypertension, myocardial infarction and cerebrovascular diseases.[13]

The treatment include anti-inflammatory (glucocorticoids) and immunosuppressive drugs (cyclophosphamide, methotrexate [MTX] and azathioprine);[14,15] in unresponsive patients, or in patients experiencing adverse drug reactions (ADRs), a treatment with TNF-α inhibitors + immunosuppressive may be used.[16-18]

In this study, we reported in 5 patients with acute TA the effects of a sequential treatment with prednisone for 5 or 7 months and then with TNF-α inhibitors for 12 months on both clinical and laboratory parameters.

MATERIALS AND METHODS

In an open label, parallel groups, double clinical centers study, conducted during the period between January 2009 and December 2012 we enrolled patients with clinical, laboratory (erythrocyte sedimentation rate [ESR] >30 mm/h and CRP; >5 mg/L) and radiological findings of acute TA. Angiography and echo-color Doppler were used to evaluate the presence and the site of vessel lesions. The disease activity was determined using the Birmingham Vascular Activity Score.[19]

This study was approved by the Institutional Review Board - Independent Ethics Committee of Interuniversity Center of Phlebolymphology - International Research and Educational Program in Clinical and Experimental Biotechnology - Headquarters at University Magna Graecia of Catanzaro and before the beginning of the study, all participants were informed about the aim, procedures, risks and benefits of the study and the they provided a written informed consent. At the time of admission and during the follow-up, a blood sample was taken from each patient in order to evaluate the plasma levels of ESR, CRP, MMP-9 and NGAL. The levels of MMPs and NGAL were evaluated through Elisa test in agreement with our previous papers.[9,20,21]

The enrolled patients received a sequential treatment with prednisone for 5 or 7 months and then with adalimumab (ADA) + MTX or infliximab (IFX) + MTX for 12 months. Follow-up was performed every month and the development of ADRs were evaluated in agreement with our previous study.[22-31]

This study may be considered to be exploratory; therefore we did not determine a power calculation.

RESULTS

During the study period, 5 new patients, of whom 4 were females and 1 male (median age 36), with acute TA were enrolled [Table 1] and signed the informed consent. Clinical evaluation documented that patients lamented fatigue, myalgia and arthralgia with a pain score of 8, measured through the visual analog scale.

Patients were treated with Prednisone for 5 or 7 months and then with ADA + MTX for 1 year or with IFX + MTX for 1 year [Table 2].

Table 1: Demographic characteristic of each patient enrolled in this study

Case	Sex	Age	Clinical evidences at admission	Vascular lesions	Surgical procedure
1	Female	34	Carotidynia, fever, multiple pulse deficit	Stenosis of right common carotid artery (40%)	None
2	Female	35	Carotidynia, myalgia, arthralgia	Stenosis of left common carotid artery (50%)	None
3	Female	36	Asymmetric arm blood pressure, myalgia, multiple pulse deficit	Stenosis of subclavian artery (40%)	None
4	Male	38	Lower limb rest pain, fever, multiple pulse deficit, myalgia, arthralgia	Stenosis of subclavian artery (30%) Abdominal aortic aneurysm (diameter 3.5 cm) Stenosis of right superficial femoral artery (80%)	Femoropopliteal by pass with autologous saphenous vein graft
5	Female	40	Carotidynia, fever, multiple pulse deficit (relapse)	Stenosis of left common carotid artery (50%)	None

Table 2: Protocol treatment used in this study

Patients	1	2	3	4	5
Prednisone	50 mg/os for 1 month 20 mg/os for 1 month 10 mg/os for 5 months	50 mg/os for 1 month 20 mg/os for 1 month 10 mg/os for 5 months	50 mg/os for 1 month 20 mg/os for 1 month 10 mg/os for 3 months	50 mg/os for 1 month 20 mg/os for 1 month 10 mg/os for 3 months	
ADA	40 mg sc/2 weeks for 1 year		40 mg sc/2 weeks for 1 year		40 mg sc/2 weeks for 1 year
MTX	7.5 mg os/week for 1 year	7.5 mg os/week for 1 year	7.5 mg os/week for 1 year	7.5 mg os/week for 1 year	
IFX		3 mg/kg/i.v. time 0, 2 and 6 weeks and then every 8 weeks for 1 year		3 mg/kg/i.v. time 0, 2 and 6 weeks and then every 8 weeks for 1 year	

ADA=Adalimumab, MTX=Methotrexate, IFX=Infliximab

In these patients, laboratory findings revealed an improvement of inflammatory index (ESR and CRP) after 2 months from the beginning of prednisone treatment. Moreover, a complete normalization of inflammatory index was recorded 4 months after the beginning of prednisone and these values maintained steady during treatment with ADA or IFX without difference between groups [Table 3].

A patient (patient 5) with relapse, enrolled in one of our previously published study [Table 1],[14] who developed ADRs to corticosteroid and intolerance to MTX received a treatment with ADA only, with a good control of laboratory index [Table 3]. All patients also showed an improvement in their clinical conditions (VAS from 8 to 1 at the end of the study). No relapses and no ADRs occurred during the study and during the follow-up of 34 months.

In all patients (Case 1-5) ELISA findings revealed higher levels of both plasma MMP-9 (mean 140 ± 4 ng/mL) and NGAL (mean 176 ± 5 ng/mL) with respect to control patients (5 healthy volunteers: 4 F, 1 M, median age 34; MMP-9: 65 ng/mL; NGAL: 20 ng/mL). In enrolled patients (Cases 1-4) the prednisone treatment induced a time-related decrease in both plasma MMP-9 and NGAL levels [Table 4], that have been maintained in normal values during the treatment with monoclonal antibodies [Table 4].

DISCUSSION

In this study, we evaluated the effects of a different drug treatment of TA in patients with acute TA symptoms.

Till date the etiology of TA disease is enigmatic since several mechanisms such as post-infective, autoimmune, ethnic susceptibility and genetic predisposition have been postulated.[32] Oxidative stress (i.e., reactive oxygen species) and reactive nitrosative species represent a cardinal feature of inflammatory process and are involved in vascular abnormalities influencing extracellular matrix remodeling through the activation of MMPs.[8] MMPs regulate extracellular structural proteins and consequent tissue remodeling and seem to be involved in various vascular disease and related complications.[11,33] NGAL is a protein belonging to the lipocalin family, is expressed by activated neutrophils and is able to modulate the activity of MMP-9 through the activation of the NGAL/MMP-9 complex. In vessels MMPs influence the migration, proliferation and apoptosis of vascular smooth muscle, endothelial cells and inflammatory cells. NGAL plasma concentrations have been associated with vascular diseases.[34-37] In agreement with these data, in our study we enrolled 5 patients with fever myalgia, claudication intermittens; angiography documented the presence of stenosis in upper diaphragmatic vessels. Previously it has been showed that MMP-1, 3 and 9 were significantly higher in subjects with active disease when compared to those study subjects who were in remission.[33-35]

The limitation of this study is related to the very low number of patients enrolled that is related with the very low frequency of this disease.

CONCLUSION

Elisa test revealed higher levels of both plasma MMP-9 and NGAL in Takayasu patients respect to control patients. Prednisone administered for 5 months showed the same effects on both clinical and laboratory findings of a longer treatment (7 months). Moreover, we showed that both ADA and IFX administered on-label with MTX improved the inflammatory indexes inducing a significant decrease in plasma levels of CRP, ESR, MMP-9 and NGAL. The

Table 3: Laboratory findings of ESR (mm/h) and CRP (mg/L) in enrolled patients

Timetable	1	2	3	4	5
ESR (mm/h)					
Admission	120	165	138	118	124
2 months	41	55	38	48	51
4 months	24	22	27	22	25
3 months after beginning of monoclonal antibodies	25	21	23	20	21
34 months from the admission	20	21	22	24	21
CRP (mg/L)					
Admission	29	22	27	24	37
2 months	10	7	9	10	12
4 months	3	4	3.8	3.4	4.1
3 months after beginning of monoclonal antibodies	3	3.5	3.7	3.6	4
34 months from the admission	3	3.4	3.6	3.6	3.9

ESR=Normal values 0-20 mm/h, CRP=Normal values 0.5-10 mg/L. ESR=Erythrocyte sedimentation rate, CRP=C-reactive protein

Table 4: Elisa values of MMP-9 (ng/mL) and NGAL (ng/mL) in enrolled patients

Timetable	1	2	3	4	5
MMP-9 (ng/mL)					
Admission	140	145	142	144	138
2 months	100	102	98	95	108
4 months	68	70	72	68	67
3 months after beginning of monoclonal antibodies	67	71	69	66	65
34 months from the admission	67	70	68	66	64
NGAL (ng/mL)					
Admission	175	172	180	180	172
2 months	80	82	75	75	172
4 months	21	22.5	21	22	20
3 months after beginning of monoclonal antibodies	20	22	22	20	21
34 months from the admission	19	20	21	20	21

MMP-9: Control value: <65 (ng/mL), NGAL: Control value: <20 (ng/mL). MMP=Matrix metalloproteinases, NGAL=Neutrophil gelatinase-associated lipocalin

patient treated on-label with ADA in monotherapy documented a good control of symptoms and of laboratory findings. No side effects appeared during the treatments. The limitation of this study is very low number of patients due to very low frequency of this disease. In conclusion, monoclonal antibodies are able to improve the symptoms of Takayasu without the development of side effects and we suggest that treatment with prednisone for 5 months leads to a good clinical outcome, although further studies are needed to validate these data. Finally, the evaluation of MMP-9 and NGAL plasma levels could be useful during the follow-up of this disease.

REFERENCES

1. Ishihara T, Haraguchi G, Tezuka D, Kamiishi T, Inagaki H, Isobe M. Diagnosis and assessment of Takayasu arteritis by multiple biomarkers. Circ J 2013;77:477-83.
2. Kallappa Parameshwarappa S, Mandjiny N, Kavumkal Rajagopalan B, Radhakrishnan N, Samavedam S, Unnikrishnan M. Intact giant abdominal aortic aneurysm due to Takayasu arteritis. Ann Vasc Surg 2013;27:671.e11-4.
3. Seko Y. Takayasu arteritis: Insights into immunopathology. Jpn Heart J 2000;41:15-26.
4. Seko Y, Sugishita K, Sato O, Takagi A, Tada Y, Matsuo H, *et al*. Expression of costimulatory molecules (4-1BBL and Fas) and major histocompatibility class I chain-related A in aortic tissue with Takayasu's arteritis. J Vasc Res 2004;41:84-90.
5. Arnaud L, Haroche J, Mathian A, Gorochov G, Amoura Z. Pathogenesis of Takayasu's arteritis: A 2011 update. Autoimmun Rev 2011;11:61-7.
6. Newby AC. Metalloproteinase expression in monocytes and macrophages and its relationship to atherosclerotic plaque instability. Arterioscler Thromb Vasc Biol 2008;28:2108-14.
7. Parakh R, Yadav A. Takayasu's arteritis: An Indian perspective. Eur J Vasc Endovasc Surg 2007;33:578-82.
8. Mahajan N, Dhawan V, Mahmood S, Malik S, Jain S. Extracellular matrix remodeling in Takayasu's arteritis: Role of matrix metalloproteinases and adventitial inflammation. Arch Med Res 2012;43:406-10.
9. de Franciscis S, Mastroroberto P, Gallelli L, Buffone G, Montemurro R, Serra R. Increased plasma levels of metalloproteinase-9 and neutrophil gelatinase-associated lipocalin in a rare case of multiple artery aneurysm. Ann Vasc Surg 2013;27:1185.e5-7.
10. Serra R, Buffone G, Falcone D, Molinari V, Scaramuzzino M, Gallelli L, *et al*. Chronic venous leg ulcers are associated with high levels of metalloproteinases-9 and neutrophil gelatinase-associated lipocalin. Wound Repair Regen 2013;21:395-401.
11. Ramos-Mozo P, Madrigal-Matute J, Vega de Ceniga M, Blanco-Colio LM, Meilhac O, Feldman L, *et al*. Increased plasma levels of NGAL, a marker of neutrophil activation, in patients with abdominal aortic aneurysm. Atherosclerosis 2012;220:552-6.
12. Nooshin D, Neda P, Shahdokht S, Ali J. Ten-year Investigation of Clinical, Laboratory and Radiologic Manifestations and Complications in Patients with Takayasu's arteritis in Three University Hospitals. Malays J Med Sci 2013;20:44-50.
13. Clinical and pathological studies of aortitis syndrome. Committee report. Jpn Heart J 1968;9:76-87.
14. de Franciscis S, Serra R, Luongo A, Sabino G, Puzziello A. The management of Takayasu's arteritis: Personal experience. Ann Vasc Surg 2007;21:754-60.
15. Borg FA, Dasgupta B. Treatment and outcomes of large vessel arteritis. Best Pract Res Clin Rheumatol 2009;23:325-37.
16. Comarmond C, Plaisier E, Dahan K, Mirault T, Emmerich J, Amoura Z, *et al*. Anti TNF-α in refractory Takayasu's arteritis: Cases series and review of the literature. Autoimmun Rev 2012;11:678-84.
17. Mahlmann A, Pfluecke C, Ouda A, Simonis G, Weiss N, Kappert U. Combined immunosuppressive therapy including a TNF-alpha blocker induces remission in a difficult to treat patient with Takayasu arteriitis and coronary involvement. Vasa 2012;41:451-7.
18. Schmidt J, Kermani TA, Bacani AK, Crowson CS, Matteson EL, Warrington KJ. Tumor necrosis factor inhibitors in patients with Takayasu arteritis: Experience from a referral center with long-term followup. Arthritis Care Res (Hoboken) 2012;64:1079-83.
19. Luqmani RA, Bacon PA, Moots RJ, Janssen BA, Pall A, Emery P, *et al*. Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. QJM 1994;87:671-8.
20. Serra R, Grande R, Buffone G, Gallelli L, de Franciscis S. The effects of minocycline on extra cellullar matrix in patients with chronic venous leg ulcers. Acta Phlebol 2013;14:99-107.
21. de Franciscis S, Gallelli L, Battaglia L, Molinari V, Montemurro R, Stillitano DM, *et al*. Cilostazol prevents foot ulcers in diabetic patients with peripheral vascular disease. Int Wound J 2013 [Epub ahead of print].
22. Gallelli L, Colosimo M, Pirritano D, Ferraro M, De Fazio S, Marigliano NM, *et al*. Retrospective evaluation of adverse drug reactions induced by nonsteroidal anti-inflammatory drugs. Clin Drug Investig 2007;27:115-22.
23. Gallelli L, Colosimo M, Tolotta GA, Falcone D, Luberto L, Curto LS, *et al*. Prospective randomized double-blind trial of racecadotril compared with loperamide in elderly people with gastroenteritis living in nursing homes. Eur J Clin Pharmacol 2010;66:137-44.
24. Gallelli L, Ferreri G, Colosimo M, Pirritano D, Flocco MA, Pelaia G, *et al*. Retrospective analysis of adverse drug reactions to bronchodilators observed in two pulmonary divisions of Catanzaro, Italy. Pharmacol Res 2003;47:493-9.
25. Gallelli L, Nardi M, Prantera T, Barbera S, Raffaele M, Arminio D, *et al*. Retrospective analysis of adverse drug reactions induced by gemcitabine treatment in patients with non-small cell lung cancer. Pharmacol Res 2004;49:259-63.
26. Gallelli L, Ferreri G, Colosimo M, Pirritano D, Guadagnino L, Pelaia G, *et al*. Adverse drug reactions to antibiotics observed in two pulmonology divisions of catanzaro, Italy: A six-year retrospective study. Pharmacol Res 2002;46:395-400.
27. Gareri P, Gallelli L, Ferreri Ibbadu G, Lacava R, Russo E, De Sarro G. Melaena following Use of the Cholinesterase Inhibitor Rivastigmine. Clin Drug Investig 2005;25:215-7.
28. Scicchitano F, Giofrè C, Palleria C, Mazzitello C, Ciriaco M, Gallelli L, *et al*. Pharmacovigilance and drug safety 2011 in Calabria (Italy): Adverse events analysis. J Res Med Sci 2012;17:872-5.
29. Gallelli L, Guadagnino V, Caroleo B, Marigliano N, De Sarro GB, Izzi A. Bilateral Skin Ulceration after Injection of Pegylated Interferon-alpha-2b in a Patient with Chronic Hepatitis C. Clin Drug Investig 2003;23:615-9.
30. Rende P, Paletta L, Gallelli G, Raffaele G, Natale V, Brissa N, *et al*. Retrospective evaluation of adverse drug reactions induced by antihypertensive treatment. J Pharmacol Pharmacother 2013;4 Suppl 1:S47-50.
31. Gallelli L, Palleria C, De Vuono A, Mumoli L, Vasapollo P, Piro B, *et al*. Safety and efficacy of generic drugs with respect to brand formulation. J Pharmacol Pharmacother 2013;4 Suppl 1:S110-4.
32. Isobe M. Takayasu arteritis revisited: Current diagnosis and treatment. Int J Cardiol 2013;168:3-10.
33. Serra R, Buffone G, Costanzo G, Montemurro R, Scarcello E, Stillitano DM, *et al*. Altered metalloproteinase-9 expression as the least common denominator between varicocele, inguinal hernia and chronic venous disorders. Ann Vasc Surg 2013 [Epub ahead of print].
34. Amato B, Coretti G, Compagna R, Amato M, Buffone G, Gigliotti D, *et al*. Role of matrix metalloproteinases in non-healing venous ulcers. Int Wound J 2013 [Epub ahead of print].
35. Serra R, Grande R. Buffone G, Molinari V, Perri P, Perri A, *et al*. Extracellular matrix assessment of infected chronic leg ulcers: Role of metalloproteinases and inflammatory cytokines. Int Wound J 2014 [Epub ahead of print].
36. Serra R, Grande R, Butrico L, Buffone G, Caliò FG, Squillace A, *et al*. Effects of a new nutraceutical substance on clinical and molecular parameters in patients with chronic venous ulceration. Int Wound J 2014 [Epub ahead of print].
37. Busceti MT, Grande R, Amato B, Gasbarro V, Buffone G, Amato M, *et al*. Pulmonary Embolism, Metalloproteinases, and Neutrophil Gelatinase Associated Lipocalin. Acta Phlebol 2013;14:115-21.

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