Clinical effects of tanshinone IIA sodium sulfonate combined with trimetazidine and levocarnitine in the treatment of AVMC and its effects on serum TNF-α, IL-18 and IL-35

PENGLIANG XU[1*], LEI JI[1*], SHUANG TIAN[2] and FUMEI LI[3]

Departments of [1]Pharmacy and [2]Spinal Surgery; [3]Intravenous Drug Distribution Center, Dongying City People's Hospital, Dongying, Shandong 257091, P.R. China

Received January 12, 2018; Accepted July 23, 2018

DOI: 10.3892/etm.2018.6671

Abstract. Clinical effects of tanshinone IIA sodium sulfonate combined with trimetazidine and levocarnitine in the treatment of acute viral myocarditis (AVMC) were investigated. Eighty-six patients with AVMC treated in Dongying City People's Hospital from August 2016 to July 2017 were selected and randomly divided into control group (n=43) and observation group (n=43). Patients in control group were treated with tanshinone IIA sodium sulfonate, while those in observation group were treated with trimetazidine and levocarnitine. The curative effect and improvement in clinical symptoms were compared between the two groups of patients, and enzyme-linked immunosorbent assay (ELISA) was used to detect the levels of heart-type fatty acid-binding protein (H-FABP), creatine kinase-MB (CK-MB) and cardiac troponin I (cTnI) in patients after treatment. Besides, the changes in levels of serum tumor necrosis factor-α (TNF-α), interleukin (IL)-18 and IL-35 were detected via ELISA. The total effective rate of treatment in observation group was significantly higher than that in control group ($p<0.05$). The improvement in clinical symptoms in observation group was significantly superior to that in control group ($p<0.05$). After treatment, levels of H-FABP, CK-MB and cTnI in observation group were obviously lower than those in control group ($p<0.05$). At 3, 5 and 7 days after treatment, the levels of TNF-α and IL-18 in both groups of patients were decreased compared with those before treatment, but the level of IL-35 was increased compared with that before treatment, and changes in observation group were more significant than those in control group ($p<0.05$). Tanshinone IIA sodium sulfonate combined with trimetazidine and levocarnitine has definite curative effects in the treatment of patients with AVMC, which can alleviate myocardial injury with higher safety, and effectively mitigate the inflammatory response in patients, so it is of great clinical significance.

Correspondence to: Pengliang Xu, Department of Pharmacy, Dongying City People's Hospital, 317 Dongcheng Nanyi Road, Dongying, Shandong 257091, P.R. China
E-mail: xpldysrmyy@163.com

*Contributed equally

Key words: acute viral myocarditis, tanshinone IIA sodium sulfonate, trimetazidine, levocarnitine, tumor necrosis factor-α, interleukin-18, interleukin-35

Introduction

Acute viral myocarditis (AVMC) is one of the common clinical diseases of the cardiovascular system. It is caused by the invasion of exogenous viruses, such as coxsackievirus, herpes virus, adenovirus, and rubella virus leading to myocardial necrosis, inflammatory cell infiltration and cardiac dysfunction (1). Early clinical manifestations of AVMC are mainly heartache, chest tightness, palpitation, fatigue and cold symptoms, leading to misdiagnosis and missed diagnosis easily. With the progression of disease, arrhythmia, heart failure and even sudden death will occur if not treated in time (2). Clinically, AVMC is usually treated with light diet, nutritional support and bed rest combined with anti-infection, anti-virus and anti-arrhythmia (3). Heart-type fatty acid-binding protein (H-FABP), creatine kinase-MB (CK-MB) and cardiac troponin I (cTnI) are the markers of myocardial injury, which can reflect the degree of myocardial damage in patients with AVMC (4). In this study, AVMC patients were treated with tanshinone IIA sodium sulfonate combined with trimetazidine and levocarnitine, the curative effect was observed, and dynamic changes in tumor necrosis factor-α (TNF-α), interleukin (IL)-18 and IL-35 before and after treatment were analyzed, so as to provide a theoretical basis for the development of AVMC standardized therapeutic regimen.

Materials and methods

General data. A total of 86 patients with AVMC treated in Dongying City People's Hospital (Dongying, China) from August 2016 to July 2017 were selected. Inclusion criteria: i) Patients meeting the diagnostic criteria of AVMC (5); ii) patients with abnormality in R wave-led ST-T segment

shown in electrocardiogram for >4 days; and iii) patients who signed the written informed consent. Exclusion criteria: i) Patients with severe renal insufficiency, or a history of heart failure; and ii) patients who were allergic to experimental drugs or pregnant patients. Patients enrolled were divided into control group (n=43) and observation group (n=43) using a random number table. There were no statistically significant differences in general data between the two groups of patients (p>0.05), and data were comparable (Table I). The study was approved by the Ethics Committee of Dongying City People's Hospital. Written informed consents were signed by the patients or their guardians.

Table I. Comparison of baseline data between the two groups of patients.

Item	Groups (n=43)		t/χ^2	P-value
	Control	Observation		
Sex (male/female)	22/21	20/23	0.046	0.829
Age (years)	40-80	40-85		
Average age (years)	38.34±5.47	39.15±5.56	0.681	0.497
BMI (kg/m^2)	22.23±3.15	22.56±3.18	0.483	0.630
NYHA grading (n, %)				
Grade II	13 (30.23)	11 (25.58)	0.058	0.810
Grade III	30 (69.77)	32 (74.42)		

BMI, body mass index.

Methods

Treatment. All patients rested in bed and received nutritional support. In control group, tanshinone IIA sodium sulfonate (approval no. NMPN H31022558; Shanghai First Biochemical & Pharmaceutical Co., Ltd., Shanghai, China) was injected intramuscularly once a day (80 mg each time). In observation group, based on the treatment in control group, trimetazidine [approval no. NMPN H20055465; Servier (Tianjin) Pharmaceutical Co., Ltd., Tianjin, China] and levocarnitine (approval no. NMPN H19990372; Shenyang First Pharmaceutical Co., Ltd., of Northeast Pharmaceutical Group, Shenyang, China) were used for treatment, in which trimetazidine tablets were taken at a draught 3 times a day (1 pill each time), and levocarnitine oral liquid was taken during meals. Adults took levocarnitine orally 3 times a day during meals (1 g each time), while the initial dose was 50 mg/kg for children, and it was slowly increased to 100 mg/kg according to the tolerance of children, but the total dose per day was not >3 g. One course of treatment lasted for 14 days for both groups of patients.

Detection of relevant indexes. After one course of treatment, 3 ml venous blood was collected from patients and centrifuged at 1,000 x g at 4˚C for 15 min to collect the supernatant. The levels of H-FABP, CK-MB and cTnI were detected via enzyme-linked immunosorbent assay (ELISA), and relevant kits were provided by RapidBio Systems, Inc. (Tucson, AZ, USA). According to the manufacturer's instructions, a microplate reader (Jiangsu Potebio Co., Ltd., Jiangsu, China) was used to read the optical density (OD) value at a wavelength of 450 nm, and the H-FABP, CK-MB and cTnI concentrations were calculated.

Before treatment and at 3, 5 and 7 days after treatment, 4 ml central venous blood was drawn from patients (fasting for >8 h) in the morning, and centrifuged at 1,000 x g at 4˚C for 20 min (centrifugal radius of 15 cm). The supernatant was taken and stored in a refrigerator at -80˚C. The levels of serum TNF-α, IL-18 and IL-35 were measured via ELISA, and relevant kits were provided by Sangon Biotech Co., Ltd. (Shanghai, China). According to the manufacturer's instructions, the microplate reader was used to read the OD value, and the TNF-α, IL-18 and IL-35 concentrations were calculated.

Evaluation indexes

Evaluation criteria of therapeutic effect. i) Remarkably effective: Clinical symptoms in patients basically disappeared after treatment, and heart rate and R wave-led ST-T segment returned to normal; ii) effective: Clinical symptoms in patients were significantly alleviated after treatment, and improvement degrees in heart rate and R wave-led ST-T segment were >80%; and iii) ineffective: clinical symptoms in patients were not improved or even aggravated after treatment (6). The improvement in symptoms, including palpitation, chest tightness, chest pain, and fatigue, in both groups of patients after treatment were observed, and the incidence rates of adverse reactions, including headache, insomnia, abnormal liver function and abnormal kidney function, in both groups after treatment were also observed.

The concentrations of H-FABP, CK-MB and cTnI in patients after treatment were detected via ELISA, and levels of TNF-α, IL-18 and IL-35 before treatment and at 3, 5 and 7 days after treatment were also detected via ELISA.

Statistical analysis. Statistical Product and Service Solutions (SPSS) 19.0 software (IBM Corp., Armonk, NY, USA) was used for data processing. Measurement data are presented as mean ± standard deviation (SD), and t-test was used. Enumeration data are presented as percentage (%), and Chi-square test was used. P<0.05 suggested that the difference was statistically significant.

Results

Comparison of curative effect between the two groups of patients. After one course of treatment, the total effective rate of treatment in observation group was significantly higher than that in control group (p<0.05) (Table II).

Comparison of improvement in symptoms between the two groups of patients after treatment. After one course of treatment, the improvement in clinical symptoms in observation group was significantly superior to that in control group (p<0.05) (Table III).

Levels of H-FABP, CK-MB and cTnI in both groups of patients after treatment. After one course of treatment, levels of H-FABP, CK-MB and cTnI in observation group were obviously lower than those in control group (p<0.05) (Table IV).

Comparison of incidence rates of adverse reactions between the two groups of patients. The incidence rates of adverse

Table II. Comparison of curative effect between the two groups of patients.

Groups	No.	Remarkably effective	Effective	Ineffective	Total effective rate
Observation	43	31 (72.09)	10 (23.26)	3 (4.65)	41 (95.35)
Control	43	19 (44.19)	14 (32.56)	10 (23.26)	33 (76.74)
χ^2			4.754		
P-value			0.029		

Table III. Comparison of improvement in symptoms between the two groups of patients after treatment.

Groups	No.	Palpitation	Chest tightness	Chest pain	Fatigue
Observation	43	1 (2.32)	0 (0.00)	0 (0.00)	1 (2.32)
Control	43	8 (18.60)	6 (13.95)	6 (13.95)	9 (20.93)
χ^2		4.468	4.479	4.479	5.545
P-value		0.035	0.034	0.034	0.019

reactions had no significant differences between the two groups of patients (p>0.05) (Table V).

Comparison of TNF-α levels between the two groups of patients. The TNF-α levels had no significant difference before treatment between the two groups of patients (p>0.05). At 3, 5 and 7 days after treatment, TNF-α levels in both groups were significantly decreased, and they were decreased more obviously in observation group than that in control group (p<0.05) (Table VI).

Comparison of IL-18 levels between the two groups of patients. The IL-18 levels had no significant difference before treatment between the two groups of patients (p>0.05). At 3, 5 and 7 days after treatment, IL-18 levels in both groups were significantly decreased, and they were decreased more obviously in observation group than that in control group (p<0.05) (Table VII).

Comparison of IL-35 levels between the two groups of patients. The IL-35 levels had no significant difference before treatment between the two groups of patients (p>0.05). At 3, 5 and 7 days after treatment, IL-35 levels in both groups were significantly increased, and they were increased more obviously in observation group than that in control group (p<0.05) (Table VIII).

Discussion

In AVMC, virus infection leads to myocardial damage in patients, thus inducing abnormal myocardial metabolism, cardiac dysfunction and other myocardial diseases (7). The mechanism of occurrence and development of AVMC is mainly as follows: Persistent virus infection in acute phase mediates the immune injury of the body, thus damaging the microvessel and myocardium of patients (8). If not treated in time, the disease will result in arrhythmia, heart failure, or

Table IV. Comparison of levels of H-FABP, CK-MB and cTnI between the two groups of patients.

Groups	No.	H-FABP (μg/ml)	CK-MB (U/ml)	cTnI (ng/ml)
Observation	43	0.83±0.14	6.67±3.23	0.08±0.02
Control	43	1.12±0.38	17.27±3.64	0.23±0.07
t-test		4.696	14.283	13.511
P-value		<0.001	<0.001	<0.001

H-FABP, heart-type fatty acid-binding protein; CK-MB, creatine kinase-MB; cTnI, cardiac troponin I.

Table V. Comparison of adverse reactions between the two groups of patients (n, %).

Groups	No.	Headache	Insomnia	Abnormal liver function	Abnormal kidney function
Observation	43	1 (2.32)	2 (4.65)	1 (2.32)	1 (2.32)
Control	43	3 (6.98)	4 (9.30)	0 (0.00)	2 (4.65)
χ^2		0.262	0.179	0.001	0.001
P-value		0.609	0.672	0.999	0.999

sudden death. There is no specific medicine in the clinical treatment of AVMC, and the main treatment method is bed rest to reduce the patient's heart burden, combined with antiviral and anti-infective treatment. At present, there is no systematic and standardized therapeutic regimen (9).

The treatment principle of AVMC is to reduce myocardial apoptosis with immune regulation through the anti-virus and symptomatic treatment, thereby improving myocardial energy metabolism and restoring normal cardiac function of patients (10). Results of this study showed that after one course of treatment, the total effective rate of treatment in observation group was significantly higher than that in control group, and the improvement effect on clinical symptoms was significantly superior to that in control group (p<0.05). This is because tanshinone IIA sodium sulfonate can act on myocardial cells and block the Ca^{2+} channel of myocardial cells, thus effectively preventing the influx of Ca^{2+}, enhancing the cell strength of patients and reducing the loss of myocardial cells, ultimately relieving the clinical symptoms of AVMC (11). Trimetazidine is a kind of piperazine compound that can optimize myocardial energy metabolism, and enhance cardiac contractility, improve myocardial oxygen supply and promote functional recovery of myocardial cells by inhibiting β oxidation of free fatty acids (12). Levocarnitine is a natural substance that can regulate lipid metabolic disorders and effectively improve cardiac dysfunction, which reduces myocardial damage by scavenging free radical accumulation, thereby promoting cardiac function recovery in AVMC patients (13).

H-FABP is a kind of low-molecular-weight cytosolic protein that exists in myocardial cells, which is often used as an index of early myocardial damage (14). CK-MB is one of the

Table VI. Comparison of TNF-α levels between the two groups of patients (ng/l).

Groups	No.	Before treatment	3 days after treatment	5 days after treatment	7 days after treatment	F	P-value
Observation	43	84.28±5.25	53.38±3.18	39.23±3.63	13.38±3.26	67.302	<0.001
Control	43	83.84±5.48	60.43±3.42	48.75±3.82	27.43±3.52	39.412	<0.001
t-test		0.380	9.899	11.846	19.203		
P-value		0.705	<0.001	<0.001	<0.001		

TNF-α, tumor necrosis factor-α.

Table VII. Comparison of IL-18 levels between the two groups of patients (pg/ml).

Groups	No.	Before treatment	3 days after treatment	5 days after treatment	7 days after treatment	F	P-value
Observation	43	165.75±7.25	92.64±4.28	66.36±3.27	23.52±3.16	69.622	<0.001
Control	43	166.82±7.57	118.56±4.63	77.47±3.36	39.56±3.74	54.652	<0.001
t-test		0.669	26.957	15.539	21.482		
P-value		0.505	<0.001	<0.001	<0.001		

IL, interleukin.

Table VIII. Comparison of IL-35 levels between the two groups of patients (pg/ml).

Groups	No.	Before treatment	3 days after treatment	5 days after treatment	7 days after treatment	F	P-value
Observation	43	46.43±3.24	86.82±3.79	117.45±5.32	137.27±7.64	71.622	<0.001
Control	43	47.62±3.58	58.67±3.23	96.35±5.25	121.67±7.23	62.735	<0.001
t-test		1.616	37.069	18.512	9.725		
P-value		0.110	<0.001	<0.001	<0.001		

IL, interleukin.

four isoforms of creatine kinase, and it is related to the muscle contraction and intracellular energy transfer, which is clinically used as an index of myocardial damage (15). Besides, cTnI is an inhibitor protein in troponin-tropomyosin regulatory complex, as well as a marker of myocardial damage (16). Results of this study showed that after treatment, levels of H-FABP, CK-MB and cTnI in observation group were significantly lower than those in control group ($p<0.05$). This is because tanshinone IIA sodium sulfonate combined with trimetazidine and levocarnitine has more obvious effects than tanshinone IIA sodium sulfonate alone, which, at the same time, can regulate the body's immunity, provide energy for myocardium, improve myocardial contractility, reduce myocardial apoptosis, increase myocardial oxygen supply, improve myocardial ischemia and hypoxia, and protect myocardium, thereby reducing the leakage of H-FABP, CK-MB and cTnI from myocardial cells and release of them into the peripheral blood. The decreased expression of H-FABP, CK-MB and cTnI indicate that myocardial damage in AVMC patients is improved to a certain extent.

TNF-α is a key inflammatory mediator core that is produced the earliest in the body, which can initiate and trigger inflammatory response and cause cascade reactions, and are of importance for neutrophil aggregation-induced damage (17). IL-18 is a pro-inflammatory cytokine that plays an important role in infection, inflammation and immune diseases (18). IL-35 is a heterodimer that is specifically produced by Treg cells, which is a cytokine necessary for Treg cells to exert the maximal inhibitory effect (19). Results of this study revealed that levels of TNF-α and IL-18 in both groups were decreased at 3, 5 and 7 days after treatment compared with those before treatment, but the level of IL-35 was increased compared with that before treatment, and changes in observation group were more obvious than those in control group ($p<0.05$). This is because when AVMC occurs, myocardium will be in ischemia and hypoxia status, so interstitial cells and myocardial cells will secrete a large amount of TNF-α, and apoptosis of myocardial cells will be induced, resulting in myocardial injury, activating inflammatory response and mediating

IL-18 secretion. At the same time, viral replication triggers programmed cell death, leading to cardiac dysfunction, destroying immune system and weakening anti-inflammatory effect, thus downregulating the expression of IL-35 (20). After treatment with tanshinone IIA sodium sulfonate combined with trimetazidine and levocarnitine, Treg cell proliferation is promoted, thus effectively regulating the body's immune response, upregulating IL-35 expression, controlling the development of AVMC, and inhibiting the inflammatory response. With the passage of treatment time, the expression of TNF-α and IL-18 are decreased, the number of inflammatory cells is decreased and the pathological injury of myocardium is improved.

In conclusion, the application of trimetazidine and levocarnitine based on the treatment of AVMC patients with tanshinone IIA sodium sulfonate can effectively improve the clinical symptoms of patients, protect the damaged myocardial cells and reduce the degree of inflammatory response. Due to the small sample size in this study, data bias was inevitable, so the sample size still needs to be expanded for further observation.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and analyzed during the study are available from the corresponding author on reasonable request.

Authors' contributions

PX and FL contributed to the detection of TNF-α, IL-18 and IL-35 expression. PX performed ELISA. LJ and ST collected and analyzed the general information of the patients. PX wrote the manuscript. All authors approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Dongying City People's Hospital (Dongying, China). Written informed consents were signed by the patients or their guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Cen Z, Guo Y, Kong Q, Zhou Q and Wu W: IL-10-producing B cells involved in the pathogenesis of Coxsackie virus B3-induced acute viral myocarditis. Int J Clin Exp Pathol 8: 830-835, 2015.
2. Yu Y, Yu Y, Liu M, Yu P, Liu G, Liu Y, Su Y, Jiang H and Chen R: Ethyl pyruvate attenuated coxsackievirus B3-induced acute viral myocarditis by suppression of HMGB1/RAGE/NF-KB pathway. Springerplus 5: 215, 2016.
3. Wu T, Chen J, Fan L, Xie W, Xu C and Wang H: Effects of Shenqi Fuzheng injection on Fas/FasL protein expression levels in the cardiomyocytes of a mouse model of viral myocarditis. Exp Ther Med 11: 1839-1846, 2016.
4. Willemsen RT, Buntinx F, Winkens B, Glatz JF, Dinant GJ; 'RAPIDA'-study team: The value of signs, symptoms and plasma heart-type fatty acid-binding protein (H-FABP) in evaluating patients presenting with symptoms possibly matching acute coronary syndrome: Background and methods of a diagnostic study in primary care. BMC Fam Pract 15: 203, 2014.
5. Cao Y, Hao L, Han CH and Zhang PY: Protective effects of Wusen Erlian granules in experimental model of viral myocarditis. Cell Biochem Biophys 71: 1129-1133, 2015.
6. Li XQ, Liu XX, Wang XY, Xie YH, Yang Q, Liu XX, Ding YY, Cao W and Wang SW: Cinnamaldehyde derivatives inhibit coxsackievirus B3-induced viral myocarditis. Biomol Ther (Seoul) 25: 279-287, 2017.
7. Cai Z, Shen L, Ma H, Yang J, Yang D, Chen H, Wei J, Lu Q, Wang DW, Xiang M, *et al*: Involvement of endoplasmic reticulum stress-mediated C/EBP homologous protein activation in coxsackievirus B3-induced acute viral myocarditis. Circ Heart Fail 8: 809-818, 2015.
8. Sisakian HS, Asatryan BA and Nahapetyan HM: Dilated cardiomyopathy: Evolution of pathogenesis concepts and potential for new therapies. N Armen Med J 9: 4-18, 2015.
9. Yang F, Mo WH, Tan BP, Wei XM and Wang H: Increased frequency of follicular helper T cells in mice viral myocarditis is relevant with anti-ANT antoantibody. Virol J 12: 20, 2015.
10. Niu L, An XJ, Tian J and Wang Y: 124 cases of clinical analysis of children with viral myocarditis. Eur Rev Med Pharmacol Sci 19: 2856-2859, 2015.
11. Zhu W, Lu Q, Wan L, Feng J and Chen HW: Sodium tanshinone II A sulfonate ameliorates microcirculatory disturbance of small intestine by attenuating the production of reactie oxygen species in rats with sepsis. Chin J Integr Med 22: 745-751, 2016.
12. Tsioufis K, Andrikopoulos G and Manolis A: Trimetazidine and cardioprotection: Facts and perspectives. Angiology 66: 204-210, 2015.
13. Fernandes S, Salta S, Bravo J, Silva AP and Summavielle T: Acetyl-L-carnitine prevents methamphetamine-induced structural damage on endothelial cells via ILK-related MMP-9 activity. Mol Neurobiol 53: 408-422, 2016.
14. Glatz JF and Renneberg R: Added value of H-FABP as plasma biomarker for the early evaluation of suspected acute coronary syndrome. Clin Lipidol 9: 205-220, 2014.
15. Banning A, Musumeci F, Penny W and Tovey JA: Reference intervals for cardiac troponin T, creatine kinase and creatine kinase-MB isoenzyme following coronary bypass graft surgery. Ann Clin Biochem 33: 561-562, 1996.
16. Sandoval Y, Smith SW, Schulz KM, Murakami MM, Love SA, Nicholson J and Apple FS: Diagnosis of type 1 and type 2 myocardial infarction using a high-sensitivity cardiac troponin I assay with sex-specific 99th percentiles based on the third universal definition of myocardial infarction classification system. Clin Chem 61: 657-663, 2015.
17. Murdaca G, Spanò F, Contatore M, Guastalla A, Penza E, Magnani O and Puppo F: Infection risk associated with anti-TNF-α agents: A review. Expert Opin Drug Saf 14: 571-582, 2015.
18. Tokmadzić VS, Tsuji Y, Bogović T, Laskarin G, Cupurdija K, Strbo N, Koyama K, Okamura H, Podack ER and Rukavina D: IL-18 is present at the maternal-fetal interface and enhances cytotoxic activity of decidual lymphocytes. Am J Reprod Immunol 48: 191-200, 2002.
19. Choi J, Leung PSC, Bowlus C and Gershwin ME: IL-35 and autoimmunity: A comprehensive perspective. Clin Rev Allergy Immunol 49: 327-332, 2015.
20. Wong CK, Leung TF, Chu IM, Dong J, Lam YY and Lam CW: Aberrant expression of regulatory cytokine IL-35 and pattern recognition receptor NOD2 in patients with allergic asthma. Inflammation 38: 348-360, 2015.