

Telmisartan reduces arrhythmias through increasing cardiac connexin43 by inhibiting IL-17 after myocardial infarction in rats

H.-Y. CHANG¹, X. LI², Y. TIAN¹

¹Department of Cardiology, First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang Province, Harbin, China

²School of Humanities and Management, Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang Province, China

Abstract. – OBJECTIVE: To observe the expressions of myocardial connexin43 (Cx43) and interleukin-17 (IL-17) in acute myocardial infarction (AMI) rats and investigate its possible mechanism of telmisartan in the prevention and treatment of arrhythmia in AMI.

MATERIALS AND METHODS: Sprague Dawley (SD) rats were selected and myocardial infarction model was established. After the successful modeling, the rats were randomly divided into three groups: Sham group, MI group, Telm group. Ventricular arrhythmias was induced by the programmed electrical stimulation at 2, 4, 8 weeks. After 8 weeks, rats were sacrificed and heart tissues were collected for immunohistochemistry and Western blot detection.

RESULTS: Telmisartan reduced the induction rate of ventricular arrhythmia after myocardial infarction in rats. Telmisartan increased the Cx43 expression while reduced the IL-17 expression in myocardial infarction in rats. Moreover, there was a negative correlation between the expressions of Cx43 and IL-17 after myocardial infarction.

CONCLUSIONS: Telmisartan can reduce the occurrence rate of malignant arrhythmias after myocardial infarction, whose mechanism may be increasing the Cx43 expression through inhibition of IL-17 expression.

Key Words:

Telmisartan, Arrhythmias, Myocardial infarction, Connexin43, IL-17.

Introduction

With the changes in modern living standard and lifestyle, the occurrence rate of cardiovascular disease has been increasing year by year. Myocardial infarction is a serious type of coronary heart disease, among which, acute myocardial infarction (AMI) has the highest mortality rate,

and it has become one of the diseases seriously threatening people's lives. Arrhythmia often occurs in the early stage of AMI, and ventricular arrhythmia is a kind of common fatal arrhythmia, but its occurrence mechanism has not been fully clear^{1,2}. In recent years, researches have shown that the expression and distribution of myocardial connexin43 (Cx43) can cause abnormalities in the gap junction structure, which is closely related to the occurrence and duration of arrhythmia³⁻⁵. The abnormal degradation and distribution of connexin can cause ventricular arrhythmias after myocardial infarction. Studies have shown that the increased angiotensin II (Ang II) may lead to the decreased expression of Cx43^{6,7}, but the Ang II receptor blockers can up-regulate the expression of Cx43 in rats with myocardial infarction and improve the remodeling of Cx43 after myocardial infarction. However, its specific mechanism still lacks the experimental evidence. It has also been found that the overexpression of interleukin-17 (IL-17) in the heart in AMI can cause the electrical remodeling of myocardial cells in mice, leading to the ventricular arrhythmias^{8,9}. Moreover, it is discovered that IL-17 can inhibit the Cx43 gene expression through activating the Jun N-terminal kinase (JNK) signaling system, thus inhibiting the gap junction intercellular communication (GJIC) mediated by gap junction^{10,11}.

With the further investigations, it is thought that the drugs can affect the structure and distribution of myocardial gap junction, which is a new field in the research on antiarrhythmic treatment. Telmisartan is a kind of blocker of Ang II receptor 1 (ATI), which has the effect of improving endothelial function¹², reversing atherosclerosis¹³, and improving myocardial remodeling and cardiac function¹⁴. There are no reports about the effect of Ang II receptor blocker (ARB) drugs on

the Cx43 remodeling and IL-17 expression after myocardial infarction. In this study, the rat model of myocardial infarction was used as the object of study to observe the expressions of myocardial Cx43 and IL-17 in AMI rats using the immunohistochemical method, and we analyzed the correlation between them and the effect of telmisartan on myocardial Cx43 and IL-17 in AMI rats. Moreover, the role and its possible mechanism of telmisartan in the prevention and treatment of arrhythmia in AMI were preliminarily investigated, so as to provide a theoretical basis for the prevention and treatment of fatal arrhythmia after AMI with ARB drugs.

Materials and Methods

Experimental Animals

Healthy adult male Sprague Dawley (SD) rats aged 16 weeks old weighing 220-250 g were selected. Experimental rats were provided by Heilongjiang University of Chinese Medicine Experimental Animal Center. The Experimental Animal Center has good indoor ventilation with illumination time of 12 h/d and room temperature at 18-25°C. Drinking and feeding were not limited. This study was approved by the Animal Ethics Committee of Heilongjiang University of Chinese Medicine Experimental Animal Center.

Establishment of Myocardial Infarction Model and Grouping

Rats were weighed and anesthetized via intraperitoneal injection of 10% chloral hydrate (40 mg/kg). At the same time, the electrocardiograph was connected, followed by electrocardiogram monitoring using limb leads. After tracheal incision, the small animal ventilator was connected with the tidal volume of 2-3 ml/100 g, inspiratory/expiratory ratio of 1:3 and respiratory rate of 60 times /min. The chest was opened through the left 3rd and 4th intercostal space to fully expose the heart, and the anterior descending branch was ligated at 1-2 mm in the inferior margin of left auricle. Electrocardiogram monitoring showed that ST segment was elevated for at least 0.5 mV and did not fall within 30 min, indicating that the myocardial infarction model was successfully established, and then the chest was sutured. All the steps in sham-operation group were the same as those in myocardial infarction model group, except for no ligation. After the successful modeling, the rats were randomly divided into three

groups: sham operation group (Sham group), acute myocardial infarction group (MI group), and telmisartan + acute myocardial infarction group (Telm group).

Ventricular Arrhythmias Induced by the Programmed Electrical Stimulation

After the intraperitoneal injection of 10% chloral hydrate (40 mg/kg) for anesthesia, rats were fixed on the table with trachea cannula, and the limb-lead electrocardiograph was connected. Then, the chest was opened to expose the heart. The bipolar needle electrode was inserted into the peripheral area around the left ventricular myocardial infarction zone and the corresponding area in Sham group as the stimulating electrode for programmed electrical stimulation to induce the ventricular arrhythmia; besides, the electrocardiogram was simultaneously recorded. The electrophysiological parameters were recorded using the BL-420F biological signal acquisition and processing system. The S1S1 perimeter of programmed electrical stimulation was 100 ms, bandwidth was 2 ms, voltage was 5 mV and the number of pulse was 8; the intensity of S2 was 5 mV, bandwidth was 2 ms, the number of pulse was 1, and the step size was -2 ms, until there was no R2 after S2, namely the refractory period or induction of ventricular arrhythmia. If the ventricular arrhythmia was not induced, the S1S2 interval was set as refractory period + 10 ms, and the premature stimulation S3 was added; then, the stimulation was decreased progressively with 2 ms until reaching the refractory period or inducing ventricular arrhythmia. Here ventricular arrhythmia referred to the continuous emergence of ventricular tachycardia (VT) or ventricular fibrillation (VF) of 6 and more than 6 wide QRS wave.

Immunohistochemistry

All paraffin specimens were sliced continuously, and each section was 4 μm thick attached to the spare slide, followed by dewaxing twice via xylene solution, hydration via gradient alcohol, microwave antigen retrieval and inactivation of endogenous peroxidase. After sealing, the primary antibody was incubated in the wet box and placed in the refrigerator at 4°C overnight. Then, the secondary antibody was incubated, followed by color development *via* diaminobenzidine (DAB), sealing *via* neutral resin and microscopic observation.

Western Blotting

About 50 mg myocardial tissues were weighed to extract the total protein, followed by bicinchoninic acid assay (BCA) protein quantification and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After the electrophoresis and membrane transfer, the primary antibody of target protein was incubated at 4°C overnight, and then the secondary antibody was incubated, followed by color development and observation via enhanced chemiluminescence (ECL).

Statistical Analysis

Statistical Product and Service Solutions (SPSS, Version X; IBM, Armonk, NY, USA) 19.0 software was used for analysis. The experimental data after semi-quantitative treatment were presented as $\bar{x} \pm s$. One-way analysis of variance was used for the comparison of means among groups, while *t*-test was used for the comparison of means between the two groups. $p < 0.05$ suggested that the difference was statistically significant.

Results

Telmisartan Reduced the Induction Rate of Ventricular Arrhythmia After Myocardial Infarction in Rats

To study the effect of telmisartan on the ventricular arrhythmia after myocardial infarction in rats, the programmed electrical stimulation was used to induce the ventricular arrhythmias. The results showed that the induction rates of ventricular arrhythmia in rats in MI group at 2 weeks, 4 weeks and 8 weeks were significantly increased compared with those in Sham group. After telmisartan intervention, the induction rates of ventricular arrhythmia in rats at 2 weeks, 4 weeks and 8 weeks were significantly decreased compared with those in MI group (Figure 1).

Effect of Telmisartan on the Cx43 Expression in Myocardial Infarction in Rats

The occurrence of fatal arrhythmia after myocardial infarction is associated with changes

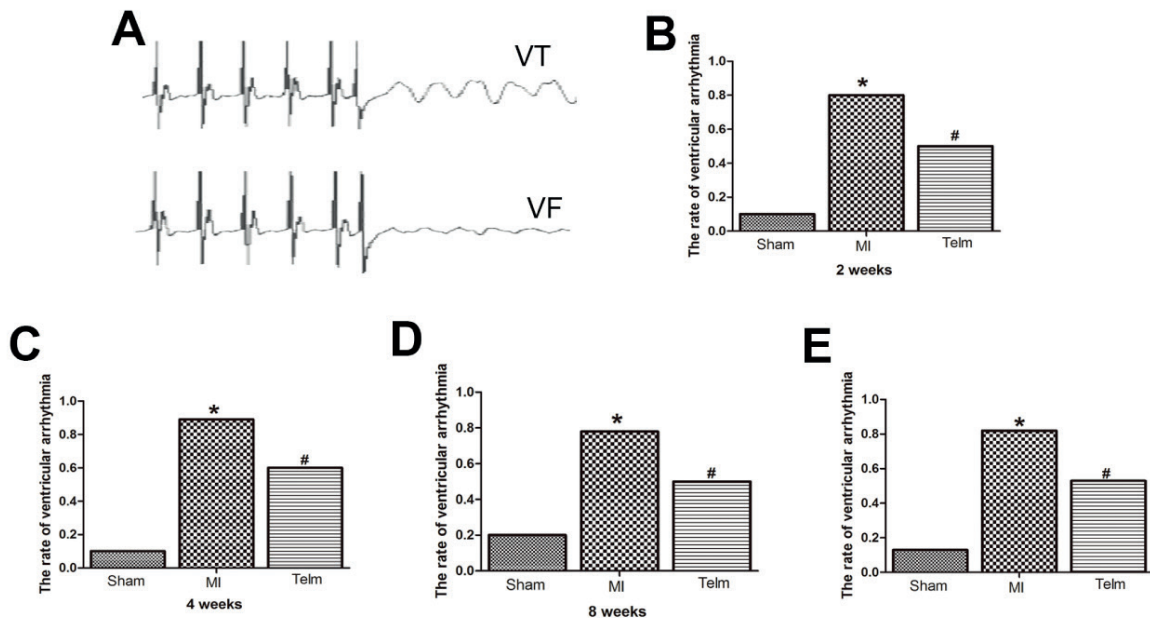


Figure 1. Telmisartan reduced the induction rate of ventricular arrhythmia after myocardial infarction in rats. (A) Ventricular tachycardia (VT) or ventricular fibrillation (VF) induced by the programmed electrical stimulation. (B) (C) (D) Analysis of the rates of ventricular arrhythmia in rats at 2 weeks, 4 weeks and 8 weeks. (E) Analysis of the rates of ventricular arrhythmia in rats in total. * $p < 0.05$ vs. Sham group, # $p < 0.05$ vs. MI group.

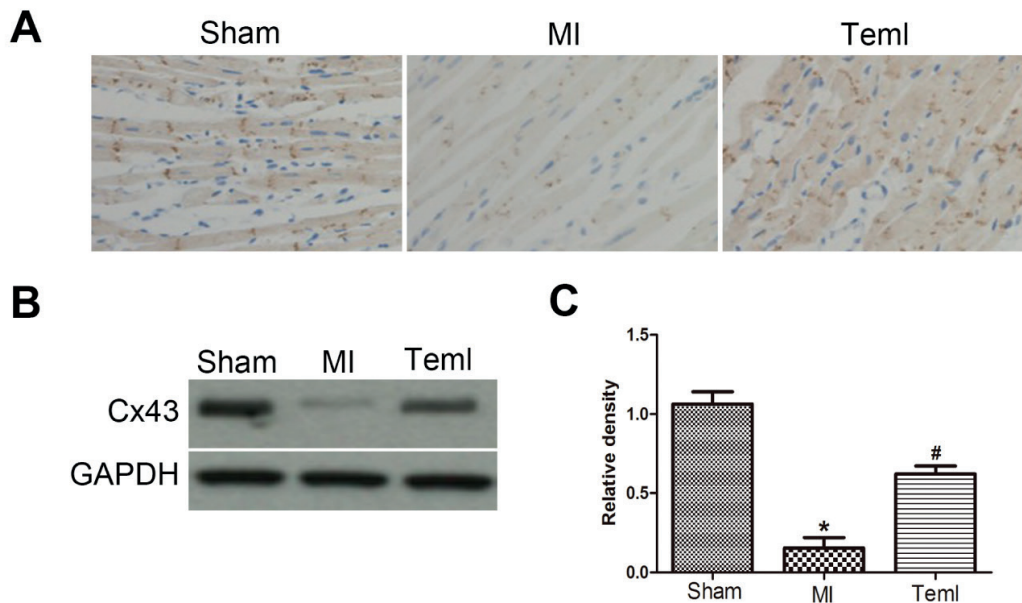


Figure 2. Effect of telmisartan on the Cx43 expression in myocardial infarction in rats. (A) Representative Immunohistochemical images of the heart 8 weeks after MI (x100). (B) Western blots analysis reveals the expression of Cx43. (C) Semi-quantitative analysis of Cx43. * $p < 0.05$ vs. Sham group, # $p < 0.05$ vs. MI group.

in gap junction channel, and Cx43 is the major connexin constituting the gap junction channel of myocardial cells. The immunohistochemical results showed that Cx43 expression in myocardial tissues of rats in Sham group was strongly positive, and its distribution was regular, mainly located in the intercalated discs. The distribution of Cx43 in MI group was disordered, and its expression was significantly decreased. After telmisartan intervention, the Cx43 remodeling in the infarcted marginal area after myocardial infarction was improved, and the electrical coupling and conduction dysfunction of the myocardial cells in the infarcted marginal area after myocardial infarction were alleviated (Figure 2A). Western blotting results further showed that the Cx43 expression was decreased after myocardial infarction, and telmisartan could increase the expression of Cx43 (Figure 2B-C).

Effect of Telmisartan on the IL-17 Expression in Myocardial Infarction in Rats

The immunohistochemical results showed that the IL-17 expression in MI group was significantly increased, while IL-17 was expressed a little in Sham group, and the IL-17 expression in myocardial cells in Teml group was significantly decreased (Figure 3A). Western blotting was used to

detect the IL-17 expression in myocardial tissues of rats in each group, and the results showed that telmisartan could significantly reduce the IL-17 expression in MI group (Figure 3B-C).

Negative Correlation Between the Expressions of Cx43 and IL-17 After Myocardial Infarction

The expressions of CX43 and IL-17 in myocardial tissues of rats in the three groups received the bivariate correlation analysis. The scatter diagrams of Cx43 and IL-17 expressions in myocardial tissues of rats in each group were drawn. The results showed that the Cx43 expression was high in Sham group, but the IL-17 expression was low, so there was no correlation between them (Figure 4A). The Cx43 expression in the myocardial tissues in the infarcted marginal area in MI group was decreased, but the IL-17 expression was increased, and they were linearly correlated. The Cx43 expression was increased and the IL-17 expression was decreased in Teml group, and they were also linearly correlated (Figure 4B-C).

Discussion

Patients with severe AMI mostly die of fatal arrhythmia or even sudden cardiac death (SCD)^{15,16}.

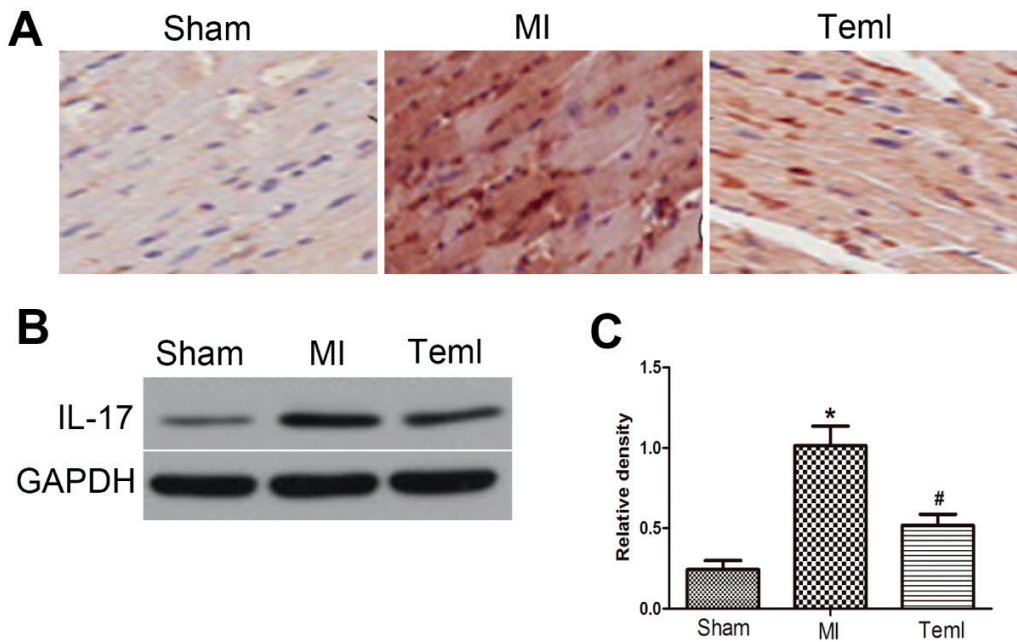


Figure 3. Effect of telmisartan on the IL-17 expression in myocardial infarction in rats. (A) Representative Immunohistochemical images of the heart 8 weeks after MI (x100). (B) Western blots analysis reveal the expression of IL-17. (C) Semi-quantitative analysis of IL-17. * $p < 0.05$ vs. Sham group, # $p < 0.05$ vs. MI group.

In this study, the AMI rat model was established and the ventricular arrhythmia was induced by programmed electrical stimulation. The results showed that the induction rate of ventricular arrhythmia in MI group was significantly increased. Studies have shown that the occurrence of fatal arrhythmia after myocardial infarction is associated with the changes in gap junction channel. Cx43 is the main connexin constituting the gap junction channel of myocardial cells, whose expression is reduced with disordered distribution after AMI, and it can lead to the electrical conduction obstacle among myocardial cells and

cause malignant arrhythmia. In this study, the AMI rat model was established to observe the changes in Cx43 expression. The results showed that the Cx43 expression in normal myocardial tissues was strongly positive and its distribution was regular, mainly located in the intercalated discs. In AMI, the distribution of Cx43 was significantly disordered and its expression was decreased. Therefore, the remodeling of Cx43 is an important molecular anatomical basis of malignant arrhythmia after myocardial infarction. The significantly decreased Cx43 expression and modified distribution pattern indicate that

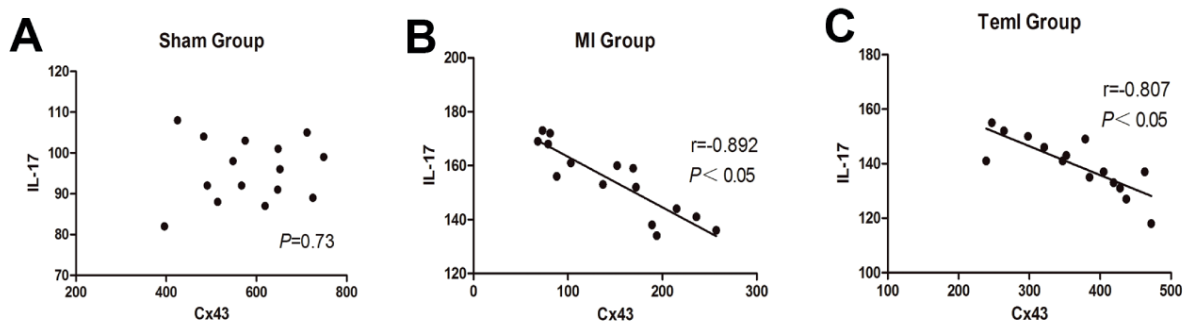


Figure 4. The correlation between the expressions of Cx43 and IL-17 after myocardial infarction. (A) There was no correlation between Cx43 and IL-17 in Sham group. (B)(C) There was a negative correlation between Cx43 and IL-17 in MI group and Teml group.

Cx43 remodeling occurs in the myocardial tissues in the infarcted marginal area of AMI rats.

Studies suggest that the important anatomical basis of malignant arrhythmia after AMI is the Cx43 remodeling. ARB drugs have been widely used in the clinical treatment of cardiovascular diseases. The clinical study argues that ARB drugs can reduce the mortality rate of myocardial infarction¹⁷⁻¹⁹, but whether the mechanism of ARB drugs in reducing the mortality rate of patients with myocardial infarction is related to the inhibition of Cx43 remodeling after AMI and reduction of malignant arrhythmia after myocardial infarction. Telmisartan is a kind of ARB that has the effect of improving endothelial function, reversing atherosclerosis and improving myocardial remodeling and cardiac function. In this work, it was found that the induction rate of ventricular arrhythmia was significantly reduced after telmisartan intervention, suggesting that telmisartan can reduce the occurrence rate of malignant arrhythmia after myocardial infarction. Moreover, the distribution of Cx43 in the myocardial infarcted marginal area was less disordered and its expression was significantly increased, indicating that telmisartan can improve the Cx43 remodeling in the myocardial infarcted marginal area of AMI rats. The results of this study showed that telmisartan could improve the Cx43 remodeling in the infarcted marginal area after myocardial infarction, thereby alleviating the electrical coupling and conduction dysfunction of myocardial cells in the infarcted marginal area after AMI, which may be one of the mechanisms of reducing the mortality rate of AMI patients. So it is speculated that the early application of telmisartan after AMI has an important clinical significance in preventing the fatal arrhythmia.

IL-17 is a kind of inflammatory cytokine with multiple biological effects, playing an important role in the inflammatory response^{20,21}. IL-17 is not expressed or very little expressed in the normal heart, and the myocardial ischemia, hemodynamic changes, increased ventricular wall tension and neuroendocrine abnormality after myocardial infarction can promote the myocardial tissues to synthesize IL-17. AMI is a kind of inflammatory process, and IL-17 plays an important role in the remodeling and final rehabilitation of heart only after AMI²². Besides, IL-17 damages the myocardial contractility mainly through changing the myocardial cell and interstitial structure. The results of this experiment showed that the expression of IL-17 was low in the myocardium tissues of normal rats, while it was significantly increased in

myocardium tissues in the infarcted marginal area of AMI rats. Telmisartan can block the pro-inflammatory effect of Ang II, and this may be because the production and release of IL-17 in local cells are reduced after telmisartan intervention, thereby inhibiting ventricular remodeling, which was proved by this experiment. The expression level of IL-17 in myocardial tissues was upregulated, promoting the remodeling process of ventricular muscle after myocardial infarction and seriously damaging the myocardial function. Studies have suggested that IL-17 can inhibit the intercellular communication among corneal fibroblasts²³ cultured *in vitro* and blood-brain barrier endothelial cells cultured *in vitro*²⁴ through inhibiting Cx43. Moreover, IL-17 can inhibit the expression of Cx43 gene and intercellular communication by activating the JNK signaling system^{25,26}. Is the mechanism of ARB drug in affecting the Cx43 remodeling after AMI related to its effect on IL-17 expression? It was found in this study that the disordered distribution of Cx43 in the infarcted marginal area in AMI rats after telmisartan intervention was alleviated, its expression was increased, and the expression of IL-17 was decreased; so, there was a linearly negative correlation between them, indicating that the mechanism of telmisartan in increasing the Cx43 expression after AMI may be related to the inhibition of IL-17 expression. However, the mechanism of telmisartan intervention in alleviating the disordered Cx43 distribution in the infarcted marginal area in AMI rats needs to be further studied.

Conclusions

Telmisartan can reduce the occurrence rate of malignant arrhythmias after myocardial infarction, whose mechanism may be increasing the Cx43 expression through inhibition of IL-17 expression.

Conflict of interest

The authors declare no conflicts of interest.

References

- 1) ERNE P, IGLESIAS JF, URBAN P, EBERLI FR, RICKLI H, SIMON R, FISCHER TA, RADOVANOVIC D. Left bundle-branch block in patients with acute myocardial infarction: presentation, treatment, and trends in outcome from 1997 to 2016 in routine clinical practice. *Am Heart J* 2017; 184: 106-113.

- 2) KURISU S, INOUE I, KAWAGOE T, ISHIHARA M, SHIMATANI Y, MITSUBA N, HATA T, NAKAMA Y, KISAKA T, KUJIMA Y. Temporary overdriving pacing as an adjunct to antiarrhythmic drug therapy for electrical storm in acute myocardial infarction. *Circ J* 2005; 69: 613-616.
- 3) NG FS, KALINDJIAN JM, COOPER SA, CHOWDHURY RA, PATEL PM, DUPONT E, LYON AR, PETERS NS. Enhancement of gap junction function during acute myocardial infarction modifies healing and reduces late ventricular arrhythmia susceptibility. *JACC Clin Electrophysiol* 2016; 2: 574-582.
- 4) FU Y, SHAO ZM, HE QZ, JIANG BQ, WU Y, ZHUANG ZG. Hsa-miR-206 represses the proliferation and invasion of breast cancer cells by targeting Cx43. *Eur Rev Med Pharmacol Sci* 2015; 19: 2091-2104.
- 5) BOULAKSIL M, BIERHUIZEN MF, ENGELEN MA, STEIN M, KOK BJ, VAN AMERSFOORTH SC, VOS MA, VAN RUIJEN HV, DE BAKKER JM, VAN VEEN TA. Spatial heterogeneity of cx43 is an arrhythmogenic substrate of polymorphic ventricular tachycardias during compensated cardiac hypertrophy in rats. *Front Cardiovasc Med* 2016; 3: 5.
- 6) HUANG QY, LI XF, LIU SP. Connexin43 and angiotensin II alterations in hearts of rats having undergone an acute exposure to alcohol. *Am J Forensic Med Pathol* 2013; 34: 68-71.
- 7) NIE W, YAN H, LI S, ZHU W, FAN F, ZHU J. Angiotensin II promotes atherogenesis through upregulating the expression of connexin 43 in dendritic cells. *Cell Mol Biol (Noisy-le-grand)* 2015; 61: 96-101.
- 8) JAFARZADEH A, ESMAEELI-NADIMI A, NOUGH H, NEMATI M, REZAYATI MT. Serum levels of interleukin (IL)-13, IL-17 and IL-18 in patients with ischemic heart disease. *Anadolu Kardiyol Derg* 2009; 9: 75-83.
- 9) SHUI XL, LIN W, MAO CW, FENG YZ, KONG JZ, CHEN SM. Blockade of IL-17 alleviated inflammation in rat arthritis and MMP-13 expression. *Eur Rev Med Pharmacol Sci* 2017; 21: 2329-2337.
- 10) BORG N, ALTER C, GORLDT N, JACOBY C, DING Z, STECKEL B, QUAST C, BONNER F, FRIEBE D, TEMME S, FLOGEL U, SCHRADER J. CD73 on T-Cells orchestrates cardiac wound healing after myocardial infarction by purinergic metabolic reprogramming. *Circulation* 2017; 136: 297-313.
- 11) REIKVAM H, RYNINGEN A, SAETERDAL LR, NEPSTAD I, FOSS B, BRUSERUD O. Connexin expression in human acute myeloid leukemia cells: Identification of patient subsets based on protein and global gene expression profiles. *Int J Mol Med* 2015; 35: 645-652.
- 12) SHANG F, ZHANG J, LI Z, ZHANG J, YIN Y, WANG Y, MARIN TL, GONGOL B, XIAO H, ZHANG YY, CHEN Z, SHYY JY, LEI T. Cardiovascular protective effect of metformin and telmisartan: reduction of PARP1 activity via the AMPK-PARP1 cascade. *PLoS One* 2016; 11: e151845.
- 13) CHAN YK, BRAR MS, KIRJAVAINEN PV, CHEN Y, PENG J, LI D, LEUNG FC, EL-NEZAMI H. High fat diet induced atherosclerosis is accompanied with low colonic bacterial diversity and altered abundances that correlates with plaque size, plasma A-FABP and cholesterol: a pilot study of high fat diet and its intervention with *Lactobacillus rhamnosus* GG (LGG) or telmisartan in ApoE^{-/-} mice. *BMC Microbiol* 2016; 16: 264.
- 14) CHANG WT, CHENG JT, CHEN ZC. Telmisartan improves cardiac fibrosis in diabetes through peroxisome proliferator activated receptor delta (PPARdelta): from bedside to bench. *Cardiovasc Diabetol* 2016; 15: 113.
- 15) HUIKURI HV, STEIN PK. Heart rate variability in risk stratification of cardiac patients. *Prog Cardiovasc Dis* 2013; 56: 153-159.
- 16) CHEN J, LI M, YU Y, WU X, JIANG R, JIN Y, LI J. Prevention of ventricular arrhythmia complicating acute myocardial infarction by local cardiac denervation. *Int J Cardiol* 2015; 184: 667-673.
- 17) GONZALEZ-CAMBEIRO MC, LOPEZ-LOPEZ A, ABU-ASSI E, RAPOSEIRAS-ROUBIN S, PENNA-GIL C, GARCIA-ACUNA J, GONZALEZ-JUANATEY R. Mortality benefit of long-term angiotensin-converting enzyme inhibitors or angiotensin receptor blockers after successful percutaneous coronary intervention in non-ST elevation acute myocardial infarction. *Rev Port Cardiol* 2016; 35: 645-653.
- 18) STRAUSS MH, HALL AS. Angiotensin receptor blockers do not reduce risk of myocardial infarction, cardiovascular death, or total mortality: further evidence for the ARB-MI paradox. *Circulation* 2017; 135: 2088-2090.
- 19) UJIL E, ROKSNOER LC, HOORN EJ, DANSER AH. From ARB to ARNI in cardiovascular control. *Curr Hypertens Rep* 2016; 18: 86.
- 20) SHAN K, PANG R, ZHAO C, LIU X, GAO W, ZHANG J, ZHAO D, WANG Y, QIU W. IL-17-triggered downregulation of miR-497 results in high HIF-1alpha expression and consequent IL-1beta and IL-6 production by astrocytes in EAE mice. *Cell Mol Immunol* 2017; 14: 909-923.
- 21) ROOS AB, STAMPFLI MR. Targeting Interleukin-17 signaling in cigarette smoke-induced lung disease: mechanistic concepts and therapeutic opportunities. *Pharmacol Ther* 2017; 178: 123-131.
- 22) JAFARZADEH A, ESMAEELI-NADIMI A, NOUGH H, NEMATI M, REZAYATI MT. Serum levels of interleukin (IL)-13, IL-17 and IL-18 in patients with ischemic heart disease. *Anadolu Kardiyol Derg* 2009; 9: 75-83.
- 23) MOLESWORTH-KENYON SJ, YIN R, OAKES JE, LAUSCH RN. IL-17 receptor signaling influences virus-induced corneal inflammation. *J Leukoc Biol* 2008; 83: 401-408.
- 24) KEBIR H, KREYMBORG K, IFERGAN I, DODELET-DEVILLERS A, CAYROL R, BERNARD M, GIULIANI F, ARBOUR N, BECHER B, PRAT A. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat Med* 2007; 13: 1173-1175.
- 25) WATANABE M, MASAKI K, YAMASAKI R, KAWANOKUCHI J, TAKEUCHI H, MATSUSHITA T, SUZUMURA A, KIRA JI. Th1 cells downregulate connexin 43 gap junctions in astrocytes via microglial activation. *Sci Rep* 2016; 6: 38387.
- 26) PATEL SA, DAVE MA, BLISS SA, GIEG-UJDA AB, BRYAN M, PLINER LF, RAMESHWAR P. Treg/Th17 polarization by distinct subsets of breast cancer cells is dictated by the interaction with mesenchymal stem cells. *J Cancer Stem Cell Res* 2014; 2014: pii: e1003.