

# Reductions in gut microbiota-derived metabolite trimethylamine N-oxide in the circulation may ameliorate myocardial infarction-induced heart failure in rats, possibly by inhibiting interleukin-8 secretion

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Received November 1, 2018; Accepted April 25, 2019

DOI: 10.3892/mmr.2019.10297

**Abstract.** Myocardial infarction (MI) is a common cause of chronic heart failure (HF). Increasing evidence has revealed that trimethylamine N-oxide (TMAO), a gut-microbiota-derived metabolite, contributes to the pathogenesis of cardiovascular disease by promoting inflammation. Elevated levels of circulating TMAO have been reported in patients following MI and were associated with unfavorable outcomes. The present study examined whether reductions in circulating TMAO could attenuate the progression of HF in rats following MI. Sprague-Dawley rats underwent coronary ligation to induce MI or a sham operation. Echocardiography confirmed MI and cardiac dysfunction one day following coronary ligation. MI and sham rats were then treated with either vehicle (tap water) or 1.0% 3,3-dimethyl-1-butanol (DMB, a trimethylamine formation inhibitor) in tap water, for 8 weeks. At the end of the experiment, TMAO plasma levels were markedly elevated in vehicle-treated MI rats compared with vehicle-treated sham rats; however, TMAO plasma levels were reduced in DMB-treated MI rats compared with vehicle-treated MI rats. Both MI groups exhibited cardiac hypertrophy, lung congestion, left ventricular remodeling and impaired cardiac function, according to the results of anatomical analysis, echocardiography and left ventricular hemodynamics; however, these manifestations of MI-induced HF were significantly improved in DMB-treated MI rats compared with vehicle-treated MI rats. The plasma levels of the chemokine interleukin (IL)-8, and cardiac expression of IL-8 and its receptors were significantly increased in

vehicle-treated MI rats compared with vehicle-treated sham rats; however, these were normalized in DMB-treated MI rats. In addition, elevated TMAO plasma level was positively correlated with increased IL-8 plasma level in MI groups. Notably, DMB treatment of sham rats also reduced plasma TMAO, but did not alter other parameters. These results indicated that reducing circulating TMAO may ameliorate the development of chronic HF following MI in rats, potentially by inhibiting IL-8 secretion. The results from the present study suggested that inhibition of TMAO synthesis may be considered as a novel therapeutic approach for the prevention and treatment of patients with chronic MI-induced HF.

## Introduction

Acute myocardial infarction (MI) is a common cause of chronic heart failure (HF), which is associated with impaired quality of life and unfavorable long-term outcomes in patients (1). Although renin-angiotensin-aldosterone inhibitors,  $\beta$ -blockers, statins and antiplatelet inhibitors have been widely used in the treatment of HF, the risks of morbidity and mortality in these patients remain high (1). A comprehensive understanding of the pathogenesis and mechanisms underlying HF is therefore crucial to develop novel therapeutic strategies for this disorder.

Increasing evidence reported that gut microbes have a crucial role in the pathogenesis of numerous cardiovascular diseases including HF, thrombosis and atherosclerosis (2,3). Trimethylamine N-oxide (TMAO), which is a gut-microbiota-derived metabolite produced from dietary constituents, has emerged as a key contributor to cardiovascular diseases (3). Dietary choline and phosphatidylcholine (4) can be metabolized to trimethylamine (TMA) by the gut microbiota. Subsequently, TMA is rapidly converted to TMAO by enzymes from the flavin monooxygenase (FMO) family, notably FMO3 in the liver. Under physiological conditions, the kidneys rapidly clear circulating TMAO via urinary excretion (4). Changes in the composition of the intestinal microbiota known as dysbiosis, or impaired renal function can lead to increased TMAO synthesis (5,6). Clinical studies have reported an association between increased circulating TMAO and increased risk of adverse cardiovascular outcomes, including heart attack, stroke and risk of mortality (2,3,7).

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**Key words:** myocardial infarction, heart failure, trimethylamine N-oxide, interleukin-8, inflammation

Experimental studies demonstrated that increased circulating TMAO worsens pressure-overload-induced HF in mice (8), induces cardiac hypertrophy and fibrosis in rats with transverse aortic constriction (9), and contributes to cardiac dysfunction in mice with Western-diet-induced obesity (10). In addition, circulating TMAO is elevated in patients hospitalized with MI and is associated with poorer prognosis, but not with Global Registry of Acute Coronary Events scores or other biomarkers of coronary artery disease, including copeptin and natriuretic peptide, proenkephalin, mid-regional proadrenomedullin and pro-substance P (7). Elevated circulating TMAO has been suggested to contribute to cardiac dysfunction and fibrosis by promoting the inflammatory response in high-fat-diet-induced obesity (10).

MI is associated with systemic and cardiac inflammation, as evidenced by increased proinflammatory cytokines and chemokines (11). Although the inflammatory response is critical for proper infarct healing, excessive inflammation has been associated with progressive HF and adverse outcomes (11). Recent studies reported that circulating interleukin (IL)-8, which is a prototypical chemokine primarily involved in the recruitment and activation of neutrophils and monocytes, is elevated and associated with large infarct size, impaired recovery of cardiac function and adverse clinical outcome in patients with MI (12,13). The biological effects of IL-8 are mediated by two the cell surface receptors CXC chemokine receptor (CXCR)1 and CXCR2 (14).

The present study examined whether reductions in circulating TMAO could improve heart function in rats with MI-induced HF, and whether this potential effect could be associated with decreased IL-8 secretion.

## Materials and methods

**Animals.** A total of 36 male Sprague-Dawley rats (age, 6 weeks; weight, 200–250 g) were obtained from the Beijing Laboratory Animal Research Center. All were housed at a controlled temperature, under a 12-h light/dark cycle, and had access to standard rat chow and water that were provided *ad libitum*. All experimental procedures were approved by the Animal Care and Welfare Committee of the Jining No. 1 People's Hospital.

**Protocol.** The rats underwent ligation of the left descending coronary artery to induce MI or a sham operation (SHAM). One day following MI or sham surgery, echocardiography was performed to assess infarct size and cardiac function; 4 rats succumbed within 24 h of MI. Animals that survived after 24 h of MI and sham animals were assigned to the following groups: i) MI rats treated with vehicle (VEH; MI + VEH; n=9); ii) MI rats treated with the TMA formation inhibitor 3,3-dimethyl-1-butanol (DMB; MI + DMB; n=7); iii) sham rats treated with vehicle (SHAM + VEH; n=8); and iv) sham rats treated with DMB (SHAM + DMB; n=8). These animals were treated with either VEH (tap water) or 1.0% DMB (Sigma-Aldrich; Merck KGaA) in drinking water for 8 weeks. The dose of DMB used in the present study has been demonstrated to effectively inhibit TMA formation and reduce plasma TMAO levels in rats (15). At termination, rats were weighed and cardiac function of each animal was assessed by

a second echocardiography and left ventricle (LV) catheterization for measurement of LV hemodynamics (16). The animals were then sacrificed, plasma samples were collected from trunk blood by centrifugation at 1,500 x g for 15 min at 4°C, and subsequently stored at -80°C for biochemical analysis. The heart and lungs were quickly removed, blotted and weighed. The heart was frozen in liquid nitrogen and stored at -80°C for molecular analysis. The lungs were weighed to calculate the ratio of wet lung to body weight as an index of pulmonary congestion in heart failure. The lungs were not frozen.

**Induction of MI.** MI was induced by ligation of the left anterior descending coronary artery as previously described (17). Briefly, rats were anesthetized with a mixture of ketamine and xylazine [100:10 mg/kg; intraperitoneal (ip)], intubated, ventilated with a rodent respirator, and laid on a heating pad to maintain their body temperature at 37°C. The heart was exposed via a left intercostal thoracotomy through the fourth intercostal space and the pericardium was removed to expose the left anterior descending coronary artery, which was ligated with a 5-0 polypropylene suture ~2 mm under the tip of the left atrium. Successful ligation of the coronary artery was confirmed by a sudden discoloration of the LV anterior wall. Sham animals were subjected to the same procedure without ligation of the coronary artery. The chest cavity was then closed and rats were allowed to recover from the anesthesia.

**Echocardiography.** Transthoracic echocardiography was performed to assess infarct size and cardiac function using a Sonos 5500R ultrasonograph system (Philips Medical Systems) equipped with a 15 MHz-linear-array transducer, as previously described (18,19). Rats were anesthetized in an induction chamber with 3% isoflurane and maintained with 1.5% isoflurane during echocardiography examinations, as previously described (20). Anesthetic depth was monitored by testing the response to toe pinching, and ensuring that animals had no retraction of the leg or withdrawing of the foot (21). Two-dimensional echocardiograms were obtained from the parasternal long-axis and short-axis views of the LV at the level of the papillary muscle tips. Two-dimensionally targeted M-mode echocardiograms were applied to measure LV dimensions in systole and diastole. All echocardiographic data were recorded with a magneto-optical disk for off-line analysis. The thickness of LV anterior and posterior walls and the LV dimensions at end cardiac diastole and systole were measured in a blinded manner, and the average of the three measurements for each parameter was used. Left ventricular end systolic volume (LVESV), left ventricular end diastolic volume (LVEDV), left ventricular fractional shortening (LVFS), left ventricular ejection fraction (LVEF) and left ventricular ischemic area (LVIA) were calculated as previously described (18).

**Cardiac hemodynamic measurements.** LV hemodynamics were measured using a Millar Microtip catheter (Millar Inc.) following catheterization of the right carotid artery. Briefly, rats were anesthetized with a mixture of ketamine and xylazine (100:10 mg/kg; ip). The right carotid artery was exposed and the Millar microtip catheter was inserted into the right carotid artery and advanced into the LV. After stabilization for

15 min, the heart rate, LV peak systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), and positive(+)/negative(-) change in pressure over time (dP/dt) were recorded.

**Western blotting.** The non-infarct LV tissue samples from MI rats and LV tissues samples from the sham rats were homogenized in RIPA lysis buffer (R0278, Sigma-Aldrich; Merck KGaA) containing 0.1% protease inhibitor cocktail (Sigma-Aldrich; Merck KGaA). Protein concentrations were measured with the Pierce bicinchoninic acid protein assay (Thermo Fisher Scientific, Inc.). Equal amounts of protein (30  $\mu$ g) were separated on 12% SDS-polyacrylamide gels and transferred onto polyvinylidene difluoride membranes (EMD Millipore, Temecula, CA, USA). Membranes were blocked with 5% milk in Tris-buffered saline containing 0.1% Tween-20 for 1 h at room temperature. Membranes were then incubated overnight at 4°C with rabbit polyclonal antibody against IL-8 (ab7747, 1:200; Abcam), rabbit polyclonal antibodies against IL-8 receptors CXCR1 (CXCR1, ab14936, 1:200; Abcam) and CXCR2 (ab14935, 1:200; Abcam), and mouse monoclonal antibody against  $\beta$ -actin (sc47778, 1:1,000; Santa Cruz Biotechnology, Inc.). Membranes were washed with Tris-buffered saline containing 0.1% Tween-20 and incubated with a mouse anti-rabbit horseradish peroxidase-conjugated secondary antibody (sc-2357, 1:5,000; Santa Cruz Biotechnology, Inc.) and a donkey anti-mouse horseradish peroxidase-conjugated secondary antibody (sc-2318, 1:5,000; Santa Cruz Biotechnology, Inc.) for 1 h at 4°C. Immunoreactive bands were visualized with an enhanced chemiluminescence detection system (GE Healthcare), and band densities were analyzed using ImageJ software version 1.49 (National Institutes of Health). Each band was normalized to  $\beta$ -actin.

**Circulating TMAO and IL-8 measurements.** Plasma TMAO levels were determined using an liquid chromatography-mass spectrometry (LC/MS) method that employed an Agilent 6120 LC/MS model mass spectrometer (Agilent Technologies, Inc.) and Phenomenex Kinetex Biphenyl Column (Phenomenex, Inc.) as previously described (10). Plasma IL-8 levels were measured using a rat IL-8 ELISA kit (MBS7606869, MyBioSource, Inc.).

**Statistical analysis.** All data are expressed as the means  $\pm$  standard error of the mean. All samples were analyzed in duplicate. A two-way analysis of variance followed by a Bonferroni's post hoc test was applied for statistical analysis using GraphPad Prism 7 (GraphPad Software, Inc.). Spearman's correlation analysis was used to determine the correlation between circulating TMAO and IL-8 levels.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Effects of MI and DMB treatment on circulating TMAO levels.** As presented in Fig. 1, at 8 weeks following coronary ligation (CL), MI + VEH rats exhibited markedly increases in plasma TMAO levels compared with SHAM + VEH rats. Furthermore, DMB treatment significantly reduced plasma TMAO levels not only in MI rats, but also in sham rats.

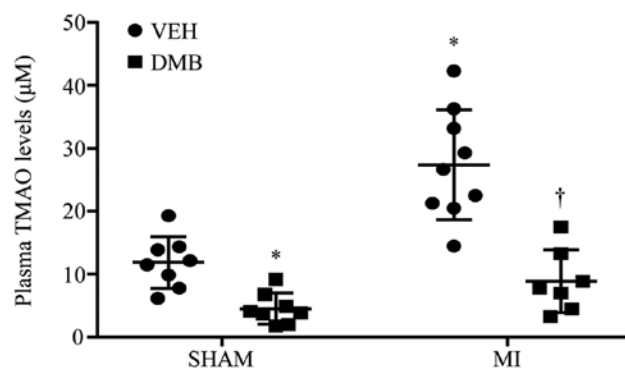


Figure 1. Effects of MI and DMB on circulating levels of TMAO. At 8 weeks following MI and DMB treatment, VEH-treated MI rats had higher circulating TMAO levels compared with VEH-treated SHAM rats. DMB treatment reduced circulating TMAO levels in both MI and SHAM rats. Data are expressed as the means  $\pm$  standard error of the mean ( $n = 7-9$  for each group). \* $P < 0.05$  vs. SHAM + VEH; † $P < 0.05$ , MI + DMB vs. MI + VEH. DMB, 3,3-dimethyl-1-butanol; MI, myocardial infarction; TMAO, trimethylamine N-oxide; VEH, vehicle.

**Effects of MI and DMB treatment on echocardiographic parameters.** Echocardiographic assessment 1 day following CL confirmed MI, as indicated by the LVIA in the two groups of MI rats. LVIA was similar for the two MI groups 1 day after CL, but was notably unaltered in the MI group 8 weeks following CL (Fig. 2B). No significant differences in LVESV (Fig. 2C), LVEDV (Fig. 2D), LVFS (Fig. 2E) and LVEF (Fig. 2F) were observed between SHAM + VEH and SHAM + DMB rats throughout the experimental protocol. Compared with the two groups of sham rats, the two groups of MI rats exhibited significant increases in LVESV and LVEDV and decreases in LVFS and LVEF both at 1 day and 8 weeks following CL. There were no significant differences in any of the echocardiographic parameters between the two MI groups 1 day after CL. In addition, 8 weeks after CL, MI + VEH rats, but not MI + DMB rats, presented further significant increases in LVESV ( $P < 0.01$ ) and LVEDV ( $P < 0.01$ ), and decreases in LVFS ( $P < 0.05$ ) and LVEF ( $P < 0.05$ ), compared with those at 1 day after CL. Notably, LVESV and LVEDV were significantly lower, while LVFS and LVEF were significantly higher in MI + DMB rats compared with MI + VEH rats 8 weeks following CL.

**Effects of MI and DMB treatment on cardiac hypertrophy, lung congestion and LV hemodynamics.** Body weight was similar across the four experimental groups (Fig. 3A) 8 weeks following CL. Compared with the SHAM + VEH rats, MI + VEH rats had significantly increased heart weight/body weight (Fig. 3B) and lung weight/body weight (Fig. 3C) ratios. MI + DMB rats had significantly lower heart weight/body weight and lung weight/body weight ratios compared with MI + VEH rats. DMB treatment had no significant effects on heart weight/body weight and lung weight/body weight ratios in the sham rats.

LV hemodynamics analyses revealed no notable differences in heart rate across the four experimental groups (Fig. 3D). LVPSP (Fig. 3E), maximum positive rate of LV developed pressure (+LVdP/dt) (Fig. 3F) and maximum negative rate of LV developed pressure (-LVdP/dt) (Fig. 3G) were significantly lower, whereas LVEDP (Fig. 3H) was higher in MI + VEH rats compared with SHAM + VEH rats. Compared with MI + VEH

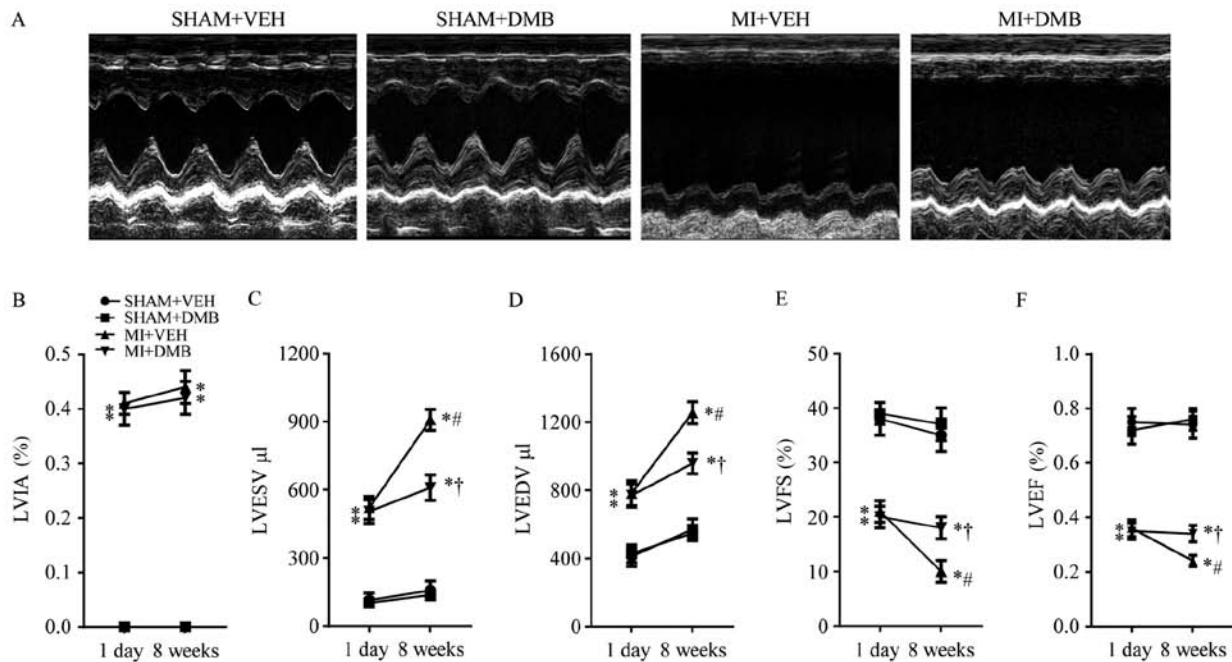


Figure 2. Effects of MI and DMB treatment on echocardiographic parameters. (A) Representative M-mode echocardiographic recordings from each group at 8 weeks following MI. (B) The percentage of LVIA remained similar at 8 weeks following MI in DMB-treated and VEH-treated MI rats. Notably, (C) LVESV and (D) LVEDV further increased, while (E) LVFS and (F) LVEF decreased further in VEH-treated MI rats but not in DMB-treated MI rats 8 weeks after MI. Data are expressed as the means  $\pm$  standard error of the mean ( $n=7-9$  for each group). \* $P<0.05$  vs. SHAM + VEH at the same time-point; † $P<0.05$ , MI + DMB vs. MI + VEH at the same time-point; # $P<0.05$ , 8 weeks vs. 1 day at the same group. DMB, 3,3-Dimethyl-1-butanol; MI, myocardial infarction; VEH, vehicle; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end diastolic volume; LVESV, left ventricular end systolic volume; LVFS, left ventricular fractional shortening; LVIA, left ventricular ischemic area.

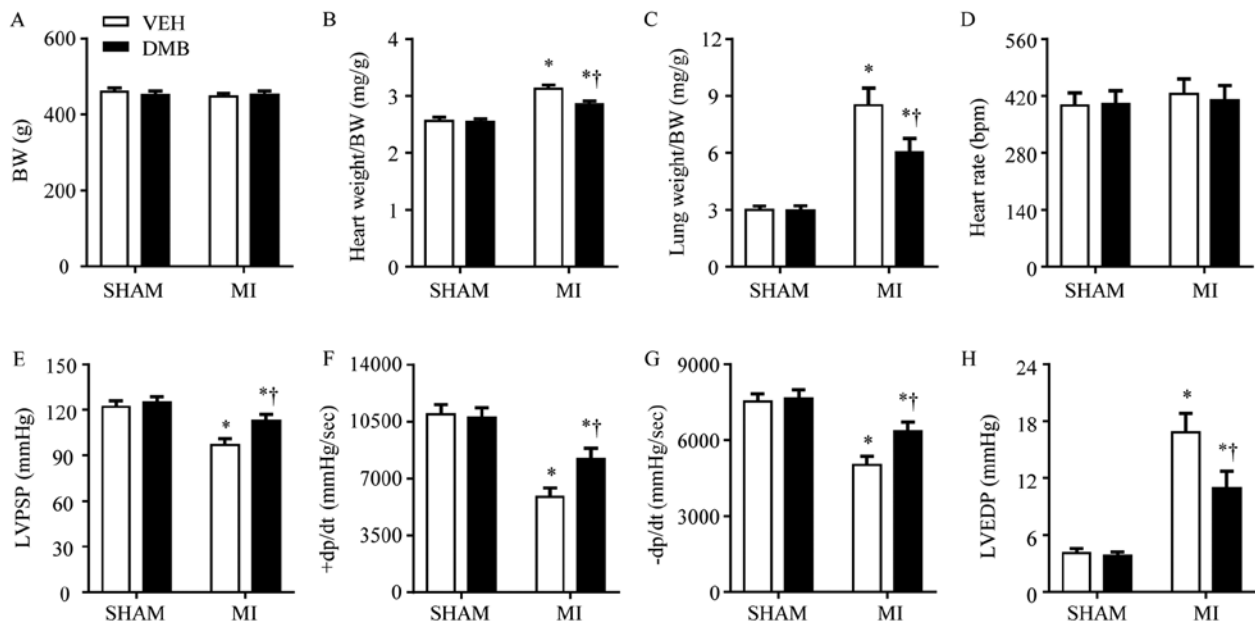


Figure 3. Effects of MI and DMB treatment on cardiac hypertrophy, lung congestion and LV hemodynamics. At 8 weeks following MI, (A) BW and (D) heart rate were similar in all groups. However, (B) heart weight/BW and (C) lung weight/BW ratios, and (H) LVEDP increased significantly, whereas (E) LVPSP, (F) +dP/dt, and (G) -dP/dt further decreased in VEH-treated MI rats than in VEH-treated SHAM rats. The factors observed in VEH-treated MI rats had improved in DMB-treated MI rats. Data are expressed as the means  $\pm$  standard error of the mean ( $n=7-9$  for each group). \* $P<0.05$  vs. SHAM + VEH; † $P<0.05$ , MI + DMB vs. MI + VEH. +dP/dt, positive change in pressure over time; -dP/dt, negative change in pressure over time; BW, body weight; DMB, 3,3-dimethyl-1-butanol; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end diastolic volume; LVESV, left ventricular end systolic volume; LVFS, left ventricular fractional shortening; LVIA, left ventricular ischemic area; LVPSP, left ventricular peak systolic pressure; MI, myocardial infarction; VEH, vehicle.

rats, MI + DMB rats had significantly increased LVPSP, +LVdP/dt, and -LVdP/dt, and decreased LVEDP. In particular,

DMB treatment had no effect on any of the hemodynamic parameters in sham rats.

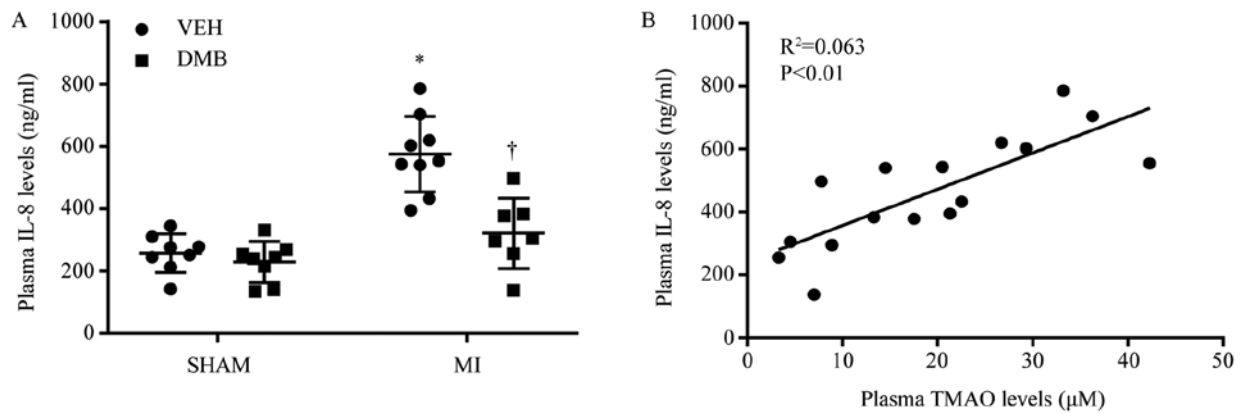


Figure 4. Effects of MI and DMB treatment on circulating IL-8 levels. At 8 weeks following MI and DMB treatment, (A) VEH-treated MI rats had elevated plasma IL-8 levels compared with VEH-treated sham rats, but decreased following treatment with DMB. (B) Elevated plasma IL-8 levels were positively correlated with increases in plasma TMAO levels in MI rats. Data are expressed as the means  $\pm$  standard error of the mean ( $n=7-9$  for each group). \* $P<0.05$  vs. SHAM + VEH; † $P<0.05$ , MI + DMB vs. MI+VEH. DMB, 3,3-dimethyl-1-butanol; IL, interleukin; MI, myocardial infarction; TMAO, trimethylamine N-oxide; VEH, vehicle.

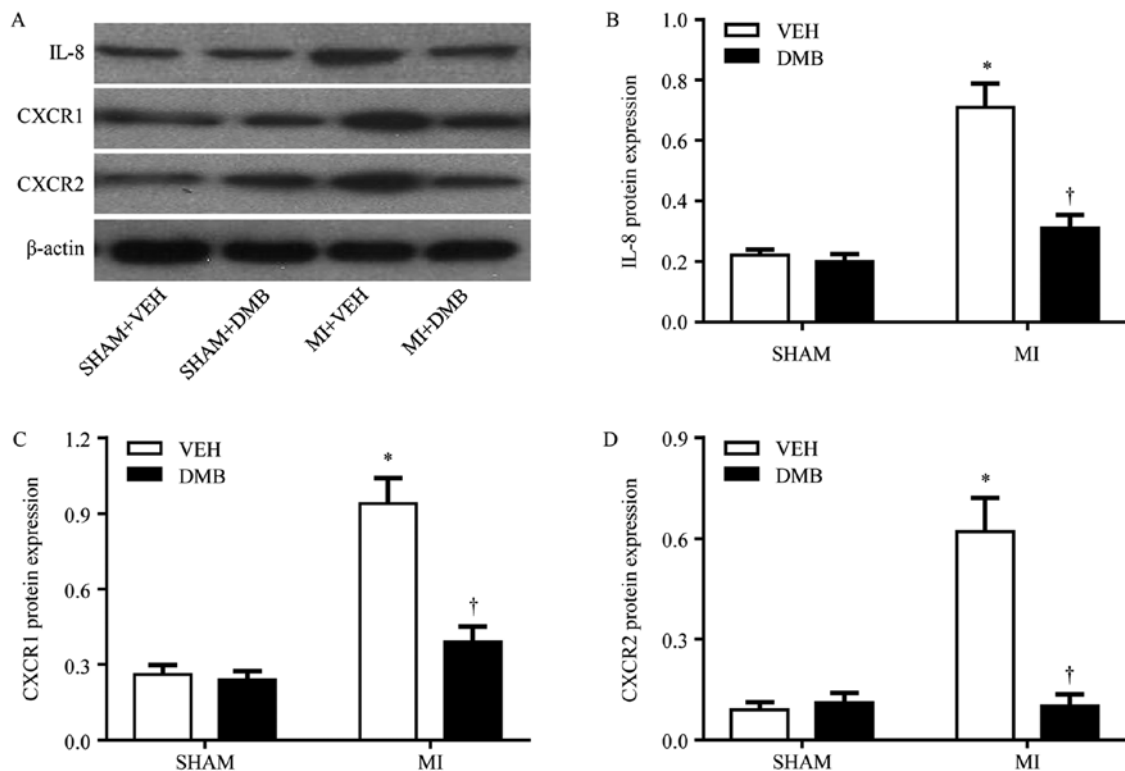


Figure 5. Effects of MI and DMB treatment on cardiac expression of IL-8 and its receptors CXCR1 and CXCR2. (A) Representative western blot for each group. The expression of (B) IL-8, and its receptors (C) CXCR1 and (D) CXCR2 was significantly increased in the non-infarct LV of MI + VEH rats, compared within the LV of SHAM + VEH rats; however, expression was normalized upon DMB treatment. Data are expressed as the means  $\pm$  standard error of the mean ( $n=7-9$  for each group). \* $P<0.05$  vs. SHAM + VEH; † $P<0.05$ , MI + DMB vs. MI + VEH. CXCR, C-X-C motif chemokine receptor; DMB, 3,3-dimethyl-1-butanol; IL, interleukin; MI, myocardial infarction; TMAO, trimethylamine N-oxide; VEH, vehicle.

**Effects of MI and DMB treatment on circulating IL-8 levels.** Compared with SHAM + VEH rats, MI + VEH rats had significantly elevated plasma IL-8 levels 8 weeks following CL (Fig. 4A). Plasma IL-8 levels were restored near to the SHAM + VEH level following treatment with DMB in MI rats. No differences in plasma IL-8 levels were observed between SHAM + DMB and SHAM + VEH rats. In addition, elevated plasma IL-8 levels were positively correlated with increased plasma TMAO levels in MI rats (Fig. 4B).

**Effects of MI and DMB treatment on expression of cardiac IL-8 and its receptors.** The expression levels of IL-8 (Fig. 5A and B) and its receptors CXCR1 (Fig. 5A and C) and CXCR2 (Fig. 5A and D) were significantly increased in the non-infarct LV of MI + VEH rats compared with the LV of SHAM + VEH rats; however, the expression of these proteins was normalized by DMB treatment. DMB treatment did not alter the expression of IL-8 (Fig. 5A and B), CXCR1 (Fig. 5A and C), and CXCR2 (Fig. 5A and D) in the LV of the sham rats.

## Discussion

The results from the present study demonstrated that treatment with DMB reduced the plasma levels of TMAO, and prevented the deterioration of cardiac remodeling and function, and lung congestion in rats following MI. Furthermore, reductions in circulating TMAO may have normalized the circulating levels of IL-8, and cardiac expression of IL-8 and its receptors in rats following MI. In addition, elevated circulating levels of IL-8 were positively correlated with corresponding increases in circulating levels of TMAO in rats following MI. Taken together, these results indicated that inhibition of TMAO synthesis may attenuate the development of HF following MI in rats, which may result from IL-8 downregulation.

Increasing evidence from experimental and clinical studies (2,8,22,23) revealed that TMAO could be an important contributor to the pathogenesis of cardiovascular diseases, including HF (3). For example, increased circulating level of TMAO has been identified as a prognostic biomarker in patients with systolic HF (2). Transverse aortic constriction in mice fed diets supplemented with either choline or TMAO induces more severe pulmonary edema, cardiac enlargement and LV ejection fraction (8). A large single-center study that examined the incremental prognostic value of circulating TMAO levels in patients with stable chronic systolic HF reported a positive correlation between the levels of TMAO and B-type natriuretic peptide (BNP), and negative correlation between TMAO levels and the estimated glomerular filtration rate (eGFR) (22). In addition, elevated circulating TMAO levels indicated increased long-term mortality independent of traditional biomarkers of risk in the HF population, including BNP and eGFR (22). Furthermore, a previous study that analyzed the predictive value of TMAO in patients with acute decompensated HF reported that circulating TMAO levels correlated with in-hospital mortality when combined with clinical risk scores that include adjustments for renal function (23). The results from the present study reported that MI + VEH rats exhibited cardiac hypertrophy, lung congestion, left ventricular remodeling and impaired cardiac function 8 weeks following CL, according to anatomical analysis, echocardiography and left ventricular hemodynamics. These data indicated that rats could develop chronic HF after MI. Furthermore, circulating TMAO levels were markedly higher in MI + VEH rats compared with SHAM + VEH rats, which was consistent with previous studies reporting that circulating TMAO levels are increased in patients with MI or chronic HF (2,7). The increase in circulating TMAO levels may be due to alterations in gut microbiota composition, which has previously been reported in mice following MI (24), or to MI-induced cardiorenal syndrome that reduces the renal capacity for excreting TMAO (5).

Reductions in circulating TMAO has been reported to attenuate the development of dietary choline-enhanced atherosclerosis (25), reduce the risk of thrombosis (26), and prevent cardiac dysfunction in mice fed with a Western diet (10). However, whether reductions in circulating TMAO have a beneficial effect on MI-induced HF remains unknown. The present study employed DMB as a drug that inhibits the formation of TMA from the gut microbiota; MI + DMB rats had reduced plasma TMAO levels compared with MI + VEH rats, which was accompanied with significant improvements in

chronic HF manifestations. These observations demonstrated that reductions in circulating TMAO may ameliorate the development of chronic HF in rats following MI.

MI is commonly accompanied with systemic and cardiac inflammation, which is characterized by increases in the production of proinflammatory cytokines and chemokines (11,27,28). In addition, the inflammatory response serves a crucial role in the development of chronic HF (11,29). It has been suggested that increased TMAO can contribute to the pathogenesis of numerous cardiovascular diseases, including obesity-induced cardiac dysfunction and aging-associated endothelial dysfunction by promoting inflammation (10,15). IL-8, a prototypical chemokine primarily involved in the recruitment and activation of neutrophils and monocytes, has recently been reported to serve a crucial role in regulating cardiac inflammation following MI (12). IL-8 is highly secreted by macrophages, endothelial cells and smooth muscle cells. In addition, circulating levels of IL-8 and cardiac expression of IL-8 and its receptors are increased in animal and human models of MI (13,30-32). Recent studies revealed that upregulated IL-8 expression is associated with large infarct size, impaired recovery of LV function and adverse clinical outcome in patients with MI (12,13); however, attenuations in IL-8 production via treatment with pterostilbene or a neutralizing monoclonal antibody is associated with decreased infarct size, and reduced myocardial apoptosis and necrosis in animals following MI (33,34). To further determine how reductions in circulating TMAO can ameliorate MI-induced HF, the present study investigated the levels of circulating IL-8, and the cardiac expression of IL-8 and its receptors. Our results demonstrated that MI + VEH rats exhibited significant increases in circulating levels of IL-8, and in the cardiac expression of IL-8, CXCR1 and CXCR2, which was consistent with previous studies (13,30-32). In particular, reductions in circulating TMAO by DMB treatment led to decreases in circulating IL-8, and cardiac expression of IL-8, CXCR1 and CXCR2 in HF rats. Furthermore, circulating TMAO was positively correlated with circulating IL-8 in HF rats. These results indicated that the effects of reducing circulating TMAO on MI-induced chronic HF may be mediated by IL-8 inhibition. Previous studies have demonstrated that IL-8 release is mediated by the proinflammatory cytokine IL-17 (35,36). This cytokine dose-dependently increases IL-8 production in smooth muscle cells (36). Furthermore, IL-17 stimulation results in the activation of NF- $\kappa$ B and MAP kinases, including p44/42 ERK, JNK and p38 MAP kinase in smooth muscle cells (35). Blockade of NF- $\kappa$ B or three MAP kinase pathways (ERK, JNK and p38 MAP kinase) reduces IL-8 synthesis by smooth muscle cells (35). These observations indicate that IL-17 induces IL-8 release via NF- $\kappa$ B and three MAP kinase pathways (ERK, JNK and p38 MAP kinase). It has been demonstrated that alterations in gut microbiota composition can induce IL-17 production (37). It is therefore possible that alterations in gut microbiota composition in rats following MI may lead to increases in circulating TMAO, which could cause IL-8 release via regulation of IL-17. In the present study, DMB treatment of MI rats inhibited TMAO synthesis, and normalized plasma levels of IL-8 and cardiac expression of IL-8 and its receptors. However, the cardiac functions of rats with MI-induced HF improved only partially, which suggested that other mechanisms, may also be involved in the pathogenesis of MI-induced HF. In addition, DMB treatment of sham rats reduced the plasma levels of TMAO, but had no effect



on IL-8 plasma levels, and the expression of IL-8, CXCR1 and CXCR2 or cardiac functions. Conversely, increased circulating levels of TMAO could induce inflammatory response in the heart, which may contribute to the development of HF following MI.

As a limitation of this study, it should be noted that the baseline plasma levels of TMAO and IL-8, and the LV hemodynamics in all rats were not determined prior to and shortly following MI induction or sham operation. Only measurements at 8 weeks post-MI induction were determined.

In conclusion, the present study demonstrated that reductions in circulating TMAO ameliorated the development of chronic HF in rats following MI, and that this beneficial effect may result from inhibiting IL-8 synthesis. The results from this study suggested that blocking the formation of the gut-microbiota-derived metabolite TMAO may be considered as a novel therapeutic approach to prevent or treat chronic HF in patients following MI.

### Acknowledgements

Not applicable.

### Funding

The present study was supported by Jining No. 1 People's Hospital.

### Availability of data and materials

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

### Authors' contributions

XL, YS and JW conceived and designed the present study. XL, YS and XZ performed the experiments. XL, YS and XZ analyzed the data. XL, YS and JW wrote the manuscript. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

All experimental procedures were approved by the Animal Care and Welfare Committee of the Jining First People's Hospital.

### Patients consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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