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Interest of colchicine in the treatment of acute myocardial infarct responsible for heart failure in a mouse model

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ABSTRACT

Background: Inflammation is deeply involved in the pathophysiology of ischemia-reperfusion (I/R) lesions and ventricular remodeling due to an acute myocardial infarction (AMI). Colchicine as a pleiotropic anti-inflammatory molecule may exert cardioprotective effects under acute ischemia. Here, we aimed to evaluate the impact of colchicine on reperfusion injury in a mouse model.

Method: Myocardial ischemia/reperfusion (I/R) injury was induced in C57BL/6 male mice, after 45 min ligation of the left coronary artery followed by reperfusion. 400 μg/kg of colchicine or the vehicle was administrated intraperitoneally (i.p.) 25 min before the reperfusion (blinded administration). Mice were sacrificed at 24 h after the acute myocardial ischemia (AMI) and the infarct size was determined. Circulating level of troponin and cytokines profile were assessed 4 h after the AMI. An echocardiography was performed in a follow-up group mice, 48 h and 8 weeks after the AMI.

Results: The infarct size was reduced in colchicine treated mice (39.8 ± 3.5% *versus* 52.9 ± 3.2%, $p < 0.05$). Troponin was significantly lower in the colchicine treated mice (7015.7 ± 1423.7 pg/mL, $n = 5$ *vs* 30,723.7 ± 7959.9 pg/mL in the placebo group, $n = 6$; $p < 0.0001$).

Fibrosis was decreased in the Colchicine group (24.51 ± 3.13% *vs* 11.38 ± 2.46%, $p = 0.03$). In the follow-up group mice ($n = 8$), there were no differences between mice treated with placebo ($n = 9$) and mice treated with colchicine ($n = 9$) regarding to cardiac remodeling parameters but outflow approximated by the ITV was higher in the colchicine group.

Conclusion: In conclusion, colchicine allowed a significant reduction of infarct size in mice, improves hemodynamic parameters and decrease cardiac fibrosis.

1. Introduction

Ischemic heart failure (HF) is a progressive disorder characterized by poor quality of life, a poor prognosis (5-year survival <50%, worse than most of common types of cancers), and a tremendous burden on health care costs [1]. In Europe and the United States ~1–2% of the entire health care budget is spent on HF. The prevalence of HF is expected to rise due to the aging population and better treatment of cardiovascular disease that precedes HF [2]. Therefore, it is mandatory to explore innovative approaches. Over the last decade, among the pathophysiological mechanisms associated with ischemic HF progression, inflammatory processes appear appealling to define new therapeutic strategies [3–5]. Indeed, after an acute myocardial infarction, inflammation could be involved at least at two levels, by worsening the infarct size at the very onset of the reperfusion, or at later stages by worsening the cardiac remodeling [4,5].

Cardiac remodeling is a crucial determinant of the clinical outcome of HF and is linked to disease progression and poor prognosis. The remodeling process is characterized by activation of "compensatory" systems, including the renin-angiotensin system (RAS) and the sympathetic nervous system (SNS). Although initially aimed at maintaining adequate circulation, over time the sustained activation of compensatory neurohormonal systems actually contributes to the adverse remodeling process leading to HF. In parallel, cardiac remodeling is

Abbreviations: AAR, area at risk; AMI, acute myocardial infarction; CAD, coronary artery disease; HF, Heart failure; IA, infarcted area; IR, ischemia-reperfusion; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; MRI, magnetic resonance imaging; RAS, renin-angiotensin system; SNS, sympathetic nervous system; STEMI, ST elevation myocardial infarction; TTC, Triphenyl-tetrazolium chloride.

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accompanied by a progressive inflammatory response characterized by inflammatory cells infiltration and pro-inflammatory cytokines, which favors fibrosis expansion [6]. As such, the severity of ischemic HF is positively correlated to the inflammatory profile [7]. Despite current treatment regimens for HF that effectively target known neurohormonal system activation, clinical outcomes remain poor. Other targets, such as cardiac fibrosis, are currently left untreated [8].

Colchicine is a mitotic spindle poison used for centuries for the treatment and prevention of gouty attacks and rheumatic complaints and is one of the oldest drugs still currently available [9]. It could exert pleiotropic anti-inflammatory effects. Especially, colchicine has direct anti-inflammatory effects [10] by inhibiting key inflammatory signaling networks as the inflammasome, pro-inflammatory cytokines and expression of adhesion molecules, preventing both local chemoattraction of inflammatory cells such as neutrophils [11] and systemic inflammation including the decrease of release of IL-1β by the neutrophils [12–14]. At the cellular level, colchicine could also exert antiarrhythmic effects [15]. In *in vivo* models, colchicine has been demonstrated to inhibit apoptosis in rats [16] and to exert indirect antifibrotic effects, by inhibiting the release of profibrotic factors [17]. Although, in a dog model subjected to a 120-min coronary artery occlusion followed by 6-h reperfusion [18], IV injection of colchicine reduce post-ischemic myocardial neutrophil accumulation, no myocardial protection could be detected in terms of infarct size. More recently colchicine has been proposed to reduce infarct size in patients [19] suggesting that colchicine could be a potential therapeutic strategy for treatment of heart failure induced by acute myocardial infarct. However, the cardioprotective impact of colchicine remains under debate.

Thus, the objective of this study was to evaluate the impact of colchicine on infarct size and cardiac remodeling in a mice model of acute myocardial infarction. We demonstrated that colchicine reduced myocardial infarct size and left ventricular remodeling was accompanied by a profound anti-inflammatory effects.

2. Method

2.1. Experimental model

Eight to ten weeks C57BL/6 male mice, were randomized into 2 groups: colchicine or placebo. Myocardial ischemia-reperfusion (IR) injuries were induced in all mice under general anesthesia with intramuscular injection of ketamine (50 mg/kg) and xylazine (10 mg/kg) and after orotracheal intubation with a 22G venous catheter for controlled ventilation (Minivent, Harvard Apparatus) with controlled stroke (10 μL/g) and frequency (150/min). After left thoracotomy and muscular dissection, ligation of the left coronary artery was performed with a 8–0 silk and a smooth catheter was applied on the artery to obtain an ischemia for 45 min. The ischemia was visually confirmed by the change in myocardial color turning into white and was followed by reperfusion obtained by the catheter removal. 400 μg/kg, 1 mg/kg and 2 mg/kg of colchicine or placebo was administrated intraperitoneally (i.p.) 25 min before the reperfusion (blinded administration). The determination of the dose is described elsewhere [20]. Muscle and cutaneous plans were sutured with silk 6–0. Mice were extubated and placed at 32 °C (RT) for 1 h. Sham-operated animals were subjected to the same surgical procedure, but the ligation remained untied. This study was approved by the local ethic committee for animal experimentation and registered by the national committee under the number CEEA-LR-12079.

Sham-operated mice underwent the same procedure without the LAD occlusion/reperfusion and treated with saline (n = 5) or colchicine (400 μg/kg, i.p.; n = 5).

2.2. Infarct size

Twenty-four hours after IR, intracardiac Evans blue injection was performed (500 μL) with a 30 G1/2 needle. Euthanasia was induced by intracardiac injection of 10% potassium chlorate.

The heart was then removed and the left ventricle (LV) was cut in 1 mm thick transverse slices, stained with Triphenyl-tetrazolium chloride 1.5% (TTC) and incubated at 37° for 2 h. The slices where then transferred in 0.9% saline serum at 4 °C and double blind analyzed with a binocular microscope (×10). The area at risk (AAR) and infarcted area (IA) were determined by computerized planimetry with ImageJ® software. The viable perfused myocardium was colored in blue, the IA in white and the ischemic viable myocardium in red. 5 slices were double blind analyzed on both side. The AAR was compounded by the IA and the ischemic but viable myocardium. The AAR/total area and IA/AAR ratios were calculated. Results are expressed in average of percentage of AAR on total area and percentage of IA on AAR, on the 5 slices.

2.3. Blood analysis

Twenty-four hours after reperfusion, a subgroup of mice was dedicated for blood analysis after intracardiac puncture under Isoflurane anesthesia. The blood was centrifuged 10 min at 5000 rpm and the serum obtained was stored at −20 °C. The myocardium injury biomarker T troponin and the major cytokines implicated in inflammatory process (IL1β, IL6, IL10, CCL2-MCP1) were measured by Multiplex (Milliplex® MAP Millipore, Billerica, MA) following the manufacture indications. Briefly, 2 plates of 96 wells were used, one for the T troponin and one for the other cytokines assay. 125 μL of serum was necessary per well, without dilution for T troponin dosage and with a 1:2 ratio dilution for the cytokines. The magnetic balls were prepared and incubated with serums overnight. The assays were triplicated for each serum with 2 negative controls by plate. The data were revealed by Luminex Multiplex assay.

2.4. Transthoracic echocardiography

A group of mice, after the same ischemia-reperfusion protocol was followed during 10 weeks.

An echocardiographic follow-up was performed 48 h, 14 days and 8 weeks after the infarction, under Isoflurane anesthesia with a maintained 36 °C body temperature and 450–500/min heart rate. The transthoracic echocardiography (Vevo2100; VisualSonics) were double blind realized, with a 40 MHz probe, TM and 2D modes were used with long and short axis parasternal views. Left ventricular ejection fraction (LVEF), left ventricular end-systolic and end-diastolic diameter, E/A profile reflecting diastolic function and aortic ITV reflecting the cardiac output were assessed.

2.5. Fibrosis study

All the mice dedicated to the long-term follow-up were sacrificed 10 weeks after ischemia-reperfusion for fibrosis study. Briefly, the heart was removed and the left ventricle was separated. The left ventricle was embedded into paraffin and 8 μm thick slices were obtained. The fibrotic area, in blue, was determined by Masson Trichrome (HT15 kit, Sigma Aldrich, France) and quantified using ImageJ. The percentage of total fibrosis area was calculated as the sum of blue-stained areas divided by total ventricular area.

2.6. Clinical sub study

A clinical trial [21] was conducted in parallel by our team and aimed to assess the impact of colchicine in post-myocardial infarction on inflammation, particularly on the peak of CRP. A sub-study was performed to investigate the impact of colchicine treatment on ventricular remodeling and to identify its predictive imaging parameters. A transthoracic echocardiography was performed in all patients included in this study. Treatment with colchicine was administered on the first day of the STEMI, for a period of 1 month at 1 mg dose per day. The left ventricular remodeling was defined as the increase in left ventricular end-diastolic volume (LVEDV) >20% at 1 month.

2.7. Statistical analysis

Statistical analyses were realized with GraphPad Prism (version 5, GraphPad software, La Jolla, CA). A Mann-Whitney test was used to compare the 2 groups of mice treated with colchicine or placebo for each analysis. The significance was fixed at $p < 0.05$. All data are expressed as percentage, mean and standard error of mean.

3. Results

3.1. Toxicity and effect of colchicine on infarct size

A preliminary phase aimed to identify the toxicity and the optimal dose of colchicine according to literature [18,20,22–24]. The ischemia-reperfusion protocol was performed with a 2 mg/kg dose of colchicine in 2 animals, 3 received 1 mg/kg of colchicine and 9 mice 400 μg/kg of colchicine. The placebo (saline serum) was administrated in 10 mice. Higher dose (≥1 mg/kg) of colchicine were toxic for 3 mice (60%) with early unexpected death (<24 h). The 400 μg/kg dose was then considered as optimal as none of them died prematurely. The two left mice treated with the higher dosage were excluded.

Twenty-six mice were sacrificed 24 h after ischemia for histological analysis, 13 in the placebo group and 13 in the colchicine group. Mean AAR/total area ratio were 52.6 ± 1.1% in the colchicine group *vs* 50.6 ± 0.8% in the placebo group ($p = 0.9$). Mean IA/AAR ratio were 39.8 ± 3.5% in the colchicine group *vs* 52.9 ± 3.2% in the placebo group ($p < 0.05$), with a significant reduction of the infarct size in the colchicine group (Fig. 1, A, B, C). After 24 h after reperfusion, the T troponin level was significantly reduced in mice treated with colchicine (7015.7 ± 1423.7 pg/mL, n = 5 *vs* 30,723.7 ± 7959.9 pg/mL in the placebo