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Matrix metalloproteinases and their tissue inhibitor after reperfused ST-elevation myocardial infarction treated with doxycycline. Insights from the TIPTOP trial☆

Giampaolo Cerisano [a,*], Piergiovanni Buonamici [a], Anna Maria Gori [b,c], Renato Valenti [a], Roberto Sciagrà [d], Betti Giusti [b], Alice Sereni [b], Silvia Raspanti [d], Paolo Colonna [e], Gian Franco Gensini [a,b,c], Rosanna Abbate [b], Richard Schulz [f], David Antoniucci [a]

[a] Department of Heart and Vessels, Careggi Hospital, Florence, Italy
[b] Department of Experimental and Clinical Medicine, University of Florence, Italy
[c] Don Carlo Gnocchi Foundation IRCCS, Florence, Italy
[d] Nuclear Medicine Unit, Department of Clinical Physiopathology — University of Florence, Careggi Hospital, Florence, Italy
[e] Division of Cardiology, Hospital Policlinico of Bari, Bari, Italy
[f] Cardiovascular Research Centre, Mazankowski Alberta Heart Institute, Departments of Pediatrics and Pharmacology, University of Alberta, Edmonton, Alberta, Canada

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ABSTRACT

Background: The TIPTOP (Early Short-term Doxycycline Therapy In Patients with Acute Myocardial Infarction and Left Ventricular Dysfunction to Prevent The Ominous Progression to Adverse Remodelling) trial demonstrated that a timely, short-term therapy with doxycycline is able to reduce LV dilation, and both infarct size and severity in patients treated with primary percutaneous intervention (pPCI) for a first ST-elevation myocardial infarction (STEMI) and left ventricular (LV) dysfunction. In this secondary, pre-defined analysis of the TIPTOP trial we evaluated the relationship between doxycycline and plasma levels of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs).

Methods: In 106 of the 110 (96%) patients enrolled in the TIPTOP trial, plasma MMPs and TIMPs were measured at baseline, and at post-STEMI days 1, 7, 30 and 180. To evaluate the remodeling process, 2D-Echo studies were performed at baseline and at 6 months. A ^{99m}Tc-SPECT was performed to evaluate the 6-month infarct size and severity.

Results: Doxycycline therapy was independently related to higher plasma TIMP-2 levels at day 7 ($p < 0.05$). Plasma TIMP-2 levels above the median value at day 7 were correlated with the 6-month smaller infarct size (3% [0%–16%] vs. 12% [0%–30%], $p = 0.002$) and severity (0.55 [0.44–0.64] vs. 0.45 [0.29–0.60], $p = 0.002$), and LV dilation (-1 ml/m^2 [from -7 ml/m^2 to 9 ml/m^2] vs. 3 ml/m^2 [from -2 ml/m^2 to 19 ml/m^2], $p = 0.04$), compared to their counterpart.

Conclusions: In this clinical setting, doxycycline therapy results in higher plasma levels of TIMP-2 which, in turn, inversely correlate with 6 month infarct size and severity as well as LV dilation.

1. Introduction

The prevention of early left ventricular (LV) remodeling during ST elevation acute myocardial infarction (STEMI) is of critical importance, especially after a large STEMI [1]. Early remodeling leads to progressive LV dilation and dysfunction which affects outcome [2], and large STEMI patients are at increased risk for LV remodeling despite primary percutaneous coronary intervention (pPCI), and the current standard of drug therapy [1]. These results highlight the need of new therapeutic strategies to limit or prevent the remodeling process.

The extracellular collagen matrix (ECM) plays a major role in post-MI remodeling, and an activation of matrix metalloproteinases (MMPs) compared to their tissue inhibitors (TIMPs) has been linked

Abbreviations: STEMI, ST elevation acute myocardial infarction; pPCI, primary percutaneous coronary intervention; ECM, extracellular collagen matrix; MMPs, matrix metalloproteinases; TIMPs, tissue inhibitors of metalloproteinases; LV, left ventricular; LVEF, left ventricular ejection fraction; SPECT, ^{99m}Tc-sestamibi-gated single-photon emission computed tomography; LVEDVi, left ventricular end-diastolic volume index; 2D Echo, two dimensional echocardiographic.

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* Corresponding author at: Department of Heart and Vessels, Careggi Hospital, Largo Brambilla 3, I-50141, Florence, Italy.
E-mail address: giampaolo.cerisano@gmail.com (G. Cerisano).

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to early ECM damage and LV dilation [3]. Accordingly, experimental studies have shown that gene deletion or pharmacological inhibition of MMP activity attenuates post-MI remodeling [4–8]. However, the structural changes of LV remodeling process have, at their base, both intra- and extracellular roots. Noteworthy, an imbalance between TIMPs and MMPs, particularly MMP-2, has been demonstrated intracellularly in isolated rat hearts subjected to ischemia and reperfusion injury [9]. This was found to be responsible for the acute contractile dysfunction and degradation of sarcomeric proteins [10–12] and, ultimately, for the infarct size [13]. Since infarct size is the major factor that promotes LV remodeling, the intracellular activation of MMPs could play a key role in this process. Unfortunately, the efforts to use synthetic MMP inhibitors in the clinical setting have failed due to the unanticipated adverse effects [14,15], and disappointing results on post-myocardial infarct remodeling process [16].

The rationale to use doxycycline to reduce post-MI remodeling is based on the fact that it is the most potent MMP inhibitor of the tetracycline class of antibiotics and exhibits MMP inhibition in vivo at blood levels lower than those required for its antibacterial effect [17]. It effectively crosses cell membranes, and accumulates preferentially at the site of tissue injury such as the damaged cardiomyocytes [18], making it appears to almost act as a smart drug [19]. Consistently, pre-clinical studies showed that doxycycline attenuates LV remodeling in a rat model of MI [20,21], prevents acute stunning in isolated rat-hearts subjected to ischaemia and reperfusion injury [12,22], and mimics the MMP-2 inhibition and infarct size reduction found with ischaemic post-conditioning [23]. Given the clinical availability and well-recognized safety profile of tetracyclines as aforementioned, doxycycline use seemed to be an ideal starting point for clinical trials in STEMI patients.

For the first time in the clinical setting, the TIPTOP (Early Short-term Doxycycline Therapy In Patients with Acute Myocardial Infarction and Left Ventricular Dysfunction to Prevent The Ominous Progression to Adverse Remodelling) trial [24] recently demonstrated that a timely short-term therapy with doxycycline is safe and able to induce a significant decrease in LV dilation, infarct size and severity in patients successfully treated with pPCI for a first STEMI and LV dysfunction.

In this secondary, pre-defined analysis of the TIPTOP trial, we set out to evaluate the time profiles of MMP and TIMP plasma levels. The results of this study may provide additional pathophysiological insights into the main study clinical findings.

2. Methods

2.1. Study design, patients and procedures

The TIPTOP trial has been already described in detail [24]. In brief, it was a prospective, phase-2, single-centre, randomized, open-label controlled trial in which 110 patients older than 18 years with acute STEMI and LV ejection fraction (LVEF) <40% were randomly assigned in a 1:1 ratio to receive doxycycline or standard care. All patients were treated with pPCI, including stenting of the infarct-related artery, and received medical therapy for STEMI and LV dysfunction in accordance with standard and recommended practice. Doxycycline (Bassado; Pfizer Italia s.r.l.) was administered at 100 mg oral dose immediately after pPCI and then every 12 h for 7 days. The antimicrobial dose we used ensures plasma levels of doxycycline similar to those obtained with doses used in preclinical studies where doxycycline was shown to be effective in inhibiting MMPs and prevented abdominal aortic aneurysm [25] and post-infarction LV remodeling [20,21]. The Σ ST-segment elevation immediately before pPCI was considered as a surrogate of the area of myocardium at risk [26].

To evaluate the remodeling process two dimensional echocardiographic (2D Echo) studies were performed at baseline (immediately after pPCI) and at 6 months with a commercially available imaging system (Philips IE-33, Amsterdam, The Netherlands). 2D Echo and Doppler studies were performed to obtain measurements of LV volumes, ejection fraction, mitral inflow E- and A-wave velocity, lateral mitral annulus E′ -and A′-wave velocity, and LV flow propagation velocity. Measurements were made using American Society of Echocardiography criteria [27]. According to the protocol of the main study [24], LV dysfunction was defined as LVEF <40%, as calculated by the on-site operator at the first echo examination in the coronary care unit immediately after pPCI. DICOM standardized images were recorded and stored on digital media, and sent to an independent, blinded, off-site core laboratory for analysis. The Core-Lab analysis was performed with a workstation-based system for 2D Echo visualization and image processing (Philips Xcelera R3.1L1, Amsterdam, The Netherlands). The mean Core-Lab inter-reader variability value for LV volumes analysis was 6.1% (ICC 0.90 with standard error 6.3 ml) and intra-reader variability was 4.1% (ICC 0.93 with standard error 3.8 ml).

^{99m}Tc-sestamibi-gated single-photon emission computed tomography (SPECT) was performed and analyzed by two experts who were unaware of the treatment group assignments, to evaluate the final infarct size and severity at 6-month follow-up. Scintigraphic acquisition began 60 min after ^{99m}Tc-sestamibi injection (740 MBq) by the use of double-head gamma-camera equipped with high-resolution collimator, with a 180° rotation arc, 32 projections, 60 s per projection, 8 frames per heart cycle, and 64 × 64 matrices. Details about the modality of acquisition and analysis of scintigraphic data have been reported elsewhere [28]. To estimate the infarct size, perfusion defects were quantified as percentage of the left ventricular wall, with the defect threshold set at 60% of peak uptake. The infarct severity, as a measure of infarct transmurality, was defined as the lowest ratio of minimal to maximal counts in the short-axis slices evaluated for infarct size; therefore, the lower the severity index the greater the infarct transmurality.

Furthermore, a coronary angiography was repeated at 6 months for the evaluation of infarct-related artery (IRA) patency. The patients were eligible for MMP/TIMP analysis when they consented for the main TIPTOP study.

The study protocol was approved by local hospital ethical committee. Doxycycline was provided directly from the local hospital pharmacy, and the manufacturer had no role in the study.

2.2. Plasma collection

Blood samples (5 cm^3) were collected from a peripheral vein into chilled ethylenediamine-tetraacetic acid tubes. The samples were centrifuged (at room temperature at 1500 *g* for 15 min) and the supernatants were removed and the plasma aliquoted and stored at −80 °C until assay. Plasma was used to measure MMP and TIMP profiles at baseline (early after PCI but prior to the administration of the first dose of doxycycline), and at post-MI days 1, 7, 30 and 180.

2.3. MMP and TIMP profiles

For this study, representative MMPs from the different MMP classes were measured, specifically the interstitial collagenase MMP-8, the gelatinases (MMP-2 and MMP-9), and MMP-7 from the matrylisin subclass. The rationale for selecting these MMP types is that they have been identified in animal studies to be altered after MI and have been associated with matrix remodeling after myocardial injury [29]. All 4 known TIMPs (TIMP-1,-2,-3 and -4) were also measured because there is a growing awareness on their role on the post-MI remodeling process [30]. The quantification of MMP and TIMP species was performed using a bead-based multiplex immunoassay. Levels of different MMPs (MMP-2, MMP-7, MMP-8, and MMP-9) and TIMPs (TIMP-1, TIMP-2, TIMP-3 and TIMP-4) were determined using the Bio-Plex suspension array system (Bio-Rad Laboratories Inc., Hercules, CA, USA) and R&D Kits (R&D System, Milan Italy) following the manufacturer's instructions. The coefficient of variation of MMP and TIMP assays were 5.8% and 6.8%, respectively [31].