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Contents

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MINIREVIEWS

ORIGINAL ARTICLE

Basic Study

CASE REPORT

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MINIREVIEWS

P2Y12-ADP receptor antagonists: Days of future and past

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Abstract

Antiplatelet therapy is the cornerstone of the therapeutic arsenal in coronary artery disease. Thanks to a better understanding in physiology, pharmacology and pharmacogenomics huge progress were made in the field of platelet reactivity inhibition thus allowing the expansion of percutaneous coronary intervention. Stent implantation requires the combination of two antiplatelet agents acting in a synergistic way. Asprin inhibit the cyclo-oxygenase pathway of platelet activation while clopidogrel is a P2Y12 adenosine diphosphate (ADP)-receptor antagonist. This dual antiplatelet therapy has dramatically improved the prognosis of stented patients. However, due to pharmacological limitations of clopidogrel (interindividual variability in its biological efficacy, slow onset of action, mild platelet reactivity inhibition) ischemic recurrences remained high following stent implantation especially in acute coronary syndrome patients. Thus, more potent P2Y12-ADP receptor inhibitors were developped including prasugrel, ticagrelor and more recently cangrelor to overcome these pitfalls. These new agents reduced the rate of thrombotic events in acute coronary syndrome patients at the cost of an increased bleeding risk. The abundance in antiplatelet agents allow us to tailor our strategy based on the thrombotic/bleeding profile of each patient. Recently, the ACCOAST trial cast a doubt on the benefit of pre treatment in non-ST segment elevation acute coronary syndrome. The aim of the present review is to summarize the results of the main studies dealing with antiplatelet therapy in stented/acute coronary syndromes patients.

Key words: Clopidogrel; Prasugrel; Ticagrelor; Acute coronary syndrome; Ticagrelor; Cangrelor

Core tip: Antiplatelet therapy in coronary artery disease has dramatically changed during the past few years. From ticlodipine to cangrelor, the present review summarizes the results of the main studies dealing with this hot topic of cardiology.

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INTRODUCTION

Allowing the expansion of percutaneous coronary intervention (PCI) and considered as the cornerstone of acute coronary syndromes (ACS) treatment, anti-platelet therapy is in large part responsible for the dramatic reduction of ischemic events observed in the past decades in ischemic heart disease patients. Indeed, dual anti-platelet therapy (DAPT) is mandatory to prevent both stent thrombosis and ischemic recurrences in stented patients. Over the past decade, from ticlodipine to clopidogrel and more recently prasugrel, ticagrelor or cangrelor, anti platelet therapy's efficacy has dramatically improved resulting in clinical benefit for our patients. This evolution is the typical example of therapeutics improvements obtained thanks to pharmacology, pharmacogenomics and clinical experience. The aim of the present review is to summarize the main results of currently available P2Y12-adenosine diphosphate (ADP) receptor antagonists.

PLATELET ACTIVATION PATHWAYS

Circulating in quiescent state, platelets can be activated through several pathways leading to the thrombus formation and growth. Following platelets' adhesion, cyclo-oxygenase 1 and 2 transform arachidonic acid into prostaglandin-H2 which is then converted in thromboxane A2 which has potent vasoconstrictor properties but also activates platelets through their TPα and TPβ receptors. Through this G-protein receptor, thromboxane A2 activates the Glycoprotein Ⅱb-Ⅲa (Gp Ⅱb-Ⅲa) receptor (final stage of platelet activation) that binds to fibrin and ensures platelets aggregation resulting in the formation of a thrombus. By inhibiting the cyclo-oxygenases, aspirin blocks the platelets' activation pathway mediated by thromboxane A2.

Another critical step of platelets' activation is mediated through ADP that is produced by red blood cells and activates platelets in an autocrine/paracrine way thanks to the P2Y1 and mainly P2Y12 receptors. The binding of ADP with its P2Y12-receptor results in a decrease of the intracellular concentration of cyclic AMP which, there again, leads to the Gp Ⅱb-Ⅲa receptor activation.

Approximately 70 agonists are involved in platelets' activation such as serotonin, thrombin, epinephrine or collagen. These other agents are not actually therapeutical targets in ischemic heart disease patients; therefore they will not be discussed in this review.

TICLODIPINE

Ticlodipine was the first commercially available P2Y12 ADP-receptor inhibitor. In the nineties, this thienopyridine has demonstrated its superiority in combination with aspirin over the gold standard therapy of that time that associated aspirin to oral anticoagulant therapy in stented patients. Schömig *et al*[1] in their study that randomized more than 500 patients demonstrated a 25% reduction in the rate of major adverse cardiovascular events (MACE) including stent thrombosis thanks to the DAPT. This benefit on ischemic events was also associated with a dramatic reduction in bleedings which are associated with poor outcome in stented patients[2].

In 1998, Leon *et al*[3] published a study that compared three anti-thrombotic regimens in stented patients: Aspirin alone, aspirin plus warfarin and aspirin in combination with ticlodipine. The primary endpoint combined death, revascularization of the target lesion, angiographically evident thrombosis and myocardial infarction at 30 d was observed in 3.6% of the aspirin group, 2.7% of the aspirin-warfarin group and 0.5% in the aspirin-ticlodipine group (P = 0.001 for the comparison of all three groups). Regarding hemorrhagic complications they occurred in respectively 1.8%, 6.2% and 5.5% of the patients (P = 0.001 for the comparison of all three groups)[3]. Bertrand *et al*[4] randomized approximately 500 stented patients to aspirin-ticlodipine or aspirin-anticoagulant therapy and unlike the previous described studies used bleedings or peripheral vascular complication as primary endpoint. Again, DAPT was superior to the former gold standard demonstrating a reduction in the primary endpoint 13.5% *vs* 21% (OR: 0.23; 95%CI: 0.05-0.91, P = 0.01). Further, DAPT reduced the rate of MACE (P = 0.01) and hospital stay (P = 0.0001) compared to the aspirin-anticoagulant therapy[4].

However, concerns were raised regarding the safety of ticlodipine. Indeed, serious hematological side effects of ticlodipine were highlighted in several studies, therefore an urgent need for a new P2Y12-inhibitor emerged.

CLOPIDOGREL

Like ticlodipine, clopidogrel belongs to the thienopyridine family. This pro-drug absorbed in the intestine required a two steps hepatic biotransformation to become active. About 85% of the absorbed clopidogrel is turned into SR26334 (an inactive metabolite) by carboxylase. The rest is metabolized by cytochrome P450 iso-enzymes in the liver. During the first step CYP2C19, CYP1A2 and CYP2B6 turn clopidogrel into 2-oxoclopidogrel which is then hydrolyzed by CYP2C19, CYP2C9 and CYP3A to become R130964, the active metabolite that irreversibly inhibits the P2Y12 ADP-receptor[5-7].

The CURE trial was the first large scale randomized study that compared the combination of aspirin-clopidogrel to aspirin alone in ACS patients. In this study that included more than 12000 patients, DAPT significantly reduced the rate of MACE (9.3% *vs* 11.4%; RR 0.80, 95%CI: 0.72-0.90, P < 0.001) at the cost of an increased in major bleedings (3.7% *vs* 2.7%; RR

1.38; $P = 0.001$)[8].

Further, the PCI CURE study randomized 2600 ACS patients treated with PCI to clopidogrel pre-treatment and long term therapy *vs* aspirin (and thienopyridine for 1 mo only following PCI). There again, clopidogrel pre-treatment and long term therapy reduced the rate of MACE by 30% (RR 0.70; 95%CI: 0.50-0.97, $P = 0.03$) without any increase in major bleedings. Consistently, clopidogrel use was associated with a lower rate of Gp Ⅱb-Ⅲa inhibitors use ($P = 0.001$)[9]. Based on these findings and on the fact that unlike ticlodipine clopidogrel is devoid of hematological side effects, DAPT combining aspirin and clopidogrel quickly became the gold standard in stented and/or ACS patients.

However, our patients are not equal before clopidogrel. Järemo *et al*[10] demonstrated in 2002 that a large inter-individual variability in response to clopidogrel exists. In fact, about 30%-40% of patients are hypo responders to clopidogrel[11]. Further, Barragan *et al*[12] correlated high on-treatment platelet reactivity (HTPR) with stent thrombosis, a finding that was later confirmed in numerous studies. Several factors may be responsible for high on-clopidogrel platelet reactivity; they can act alone or combined. Genetic polymorphism has been one of the first causes of HTPR investigated. Three independent genes have clearly been related to clopidogrel hypo responsiveness: CYP2C19 (*2), CYP3A4 and ABCB1[13-18]. Drug-drug interaction with proton pump inhibitor, but also calcium channel blocker or statin (even if the evidence level is lower for the two latest)[19,20]; clinical factors (diabetes mellitus, acute coronary syndrome and obesity) or biological factors (high platelet turnover, platelet receptors up-regulation) have also been incriminated[21-24].

Beside its inter-individual platelet reactivity, clopidogrel possesses other limitations including a slow onset of action (especially in the ACS setting) and it induced a mild platelet reactivity inhibition. Therefore, new drugs devoid of these disadvantages were developed: Prasugrel and ticagrelor.

PRASUGREL

Prasugrel is defined as a third generation thienopyridine that irreversibly inhibits platelets like clopidogrel through its P2Y12-ADP receptor. Prasugrel is also a pro-drug that requires hepatic bio-transformation to become active. Following absorption it is hydrolyzed into R-95913 (a thiolactone) by esterases[25]. Then CYP3A4, CYP2B6, CYP2C19, CYP2C9, and CYP2D6 turn it into R-138727, the active metabolite[26]. Interestingly, prasugrel active metabolite possesses similar efficacy than the active metabolite of clopidogrel suggesting that its higher potency is related to its simpler metabolism[27].

The PRINCIPLE-TIMI 44 study is a phase 2 trial that demonstrated a faster onset of action and a more potent platelet reactivity inhibition with prasugrel compared to clopidogrel in patients undergoing PCI[28].

The TRITON trial compared prasugrel to clopidogrel in 13608 ACS patients treated with PCI. This study demonstrated a 19% reduction of the primary endpoint composed of myocardial infarction, stroke, and cardiovascular deaths with prasugrel compared to clopidogrel (9.9% *vs* 12.1%; HR 0.81, 95%CI: 0.73-0.90, $P < 0.001$) at the cost of increased life-threatening bleedings (1.4% *vs* 0.9%, $P = 0.01$). Further analysis of this study revealed the lack of benefit of prasugrel in elderly (⩾ 75 years) or small weighted patients (< 60 kg) and a potential harm in patients with an history of stroke or transient ischemic attack history leading to a restriction of use in the first described populations and a contra-indication in the later[29].

It is important to keep in mind that prasugrel should only be administered in ACS patients treated with PCI, once the coronary anatomy is known, given the design of the TRITON trial that randomized patients after coronary angiography.

The TRILOGY ACS study randomized more than 7000 ACS patients medically managed (*i.e.*, without revascularization) to prasugrel or clopidogrel in this clinical setting. In this study, the rate of myocardial infarction, stroke or cardiovascular death (primary endpoint) was similar between both groups (prasugrel: 13.9%, clopidogrel: 16%; HR: 0.91, 95%CI: 0.79-1.05, $P = 0.21$). Therefore, prasugrel is not recommended in this situation[30].

More recently, the ACCOAST study compared in 4033 non-ST segment elevation ACS patients the impact of a 30 mg pre-treatment of prasugrel (a half loading-dose administered after randomization, complement being administered after PCI) to a full loading dose (60 mg) once the PCI is performed. No benefit was found to pre-treat the patient. The primary endpoint composed of myocardial infarction, stroke, death from cardiovascular causes, urgent revascularization, Gp Ⅱb/Ⅲa bailout was similar in the two groups (HR pre-treatment: 1.02, 95%CI: 0.84-1.25, $P = 0.81$) but the rate of TIMI major bleeding was higher in the pre-treatment group despite the half loading dose used before coronary angiography ($P = 0.006$)[31].

TICAGRELOR

Unlike clopidogrel or prasugrel, ticagrelor does not belong to the thienopyridine family but to the cyclopentyltriazolopyrimidine family. Divergences with previous drugs go further than this classification; indeed ticagrelor is not a pro-drug and reversibly inhibits P2Y12-ADP receptor. Ticagrelor's main active metabolite (namely AR-C124910) is formed by O-de-ethylation that depends on CYP3A4. This metabolite (also active) can reach 40% of the concentration of ticagrelor[32].

The phase 2 trial ONSET/OFFSET demonstrated the faster onset of action associated with a more potent platelet reactivity inhibition of ticagrelor compared to clopidogrel in stable patients[33].

The PLATO trial compared ticagrelor to clopidogrel in 18624 patients ACS patients and founded a significant

reduction of the rate of the primary endpoint (death from cardiovascular causes, myocardial infarction or stroke) in the ticagrelor group: 9.8% *vs* 11.7% (HR 0.84, 95%CI: 0.77-0.92, $P < 0.001$). Unlike prasugrel, ticagrelor reduced the rate of death from any causes ($P = 0.001$) compared to clopidogrel. Of note, the rate of death in the clopidogrel group of the PLATO trial was 5.9% while it was 3.2% in the clopidogrel group of the TRITON trial suggesting a lower risk population included in the latter study[29,34]. Interestingly, the benefit of ticagrelor was present whatever the method of revascularization used (PCI, CABG, none).

Concerning the safety, ticagrelor administration was associated with an increased risk of major bleedings not related to CABG ($P = 0.03$).

In the ATLANTIC study, investigators evaluated the efficacy of ticagrelor pre-treatment in 1862 STEMI patients compared to the administration of the loading dose in the cath lab. In this study, both strategies resulted in a similar efficacy[35].

CANGRELOR

Cangrelor is a non-thienopyridine intra-venous agent that reversibly inhibits P2Y12 ADP-receptor. Like ticagrelor, it does not require hepatic biotransformation to become active explaining its quick onset of action. Further, half-life of cangrelor is 3-6 min while platelets resume normal reactivity 30-60 min after discontinuation of the infusion[36]. Theoretically, cangrelor seems to be an interesting drug: A rapid onset of action, a potent platelet reactivity inhibition and a rapid reversible effect. However, despite these promising properties, the CHAMPION PCI and CHAMPION PLATFORM trials failed to demonstrate any benefit of cangrelor compared to clopidogrel in patients treated with PCI[37,38].

Later, the CHAMPION PHOENIX trial redefined periprocedural myocardial infarction and used an angiographic core lab. This study that once again compared cangrelor to clopidogrel in patients treated with PCI found a significant reduction in the rate of primary endpoint (death from any cause, myocardial infarction, ischemia driven revascularization, stent thrombosis at 48 h) in the cangrelor group (4.7% *vs* 5.9%, OR 0.79, 95%CI: 0.67-0.93, $P = 0.006$) without difference regarding severe bleedings[39].

Interestingly, the BRIDGE study confirmed a better platelet reactivity inhibition with cangrelor compared to placebo without significant difference in bleedings in ACS patients that discontinued thienopyridine before CABG[40].

Thanks to its pharmacological properties, cangrelor might be interesting in patients treated with P2Y12 inhibitors that require drug discontinuation before surgery or in unconscious patients admitted for ACS, unable to take orally administered anti platelet agents or in vomiting patients, a frequent setting during STEMI or in morphine treated patients.

CONCLUSION

Therapeutics has constantly improved over the last decades for the best of our patients. However, several debates remain regarding pre-treatment or optimal duration of DAPT in ACS patients emphasizing the importance of personalized treatment in stented patients.

REFERENCES

1 **Schömig A**, Neumann FJ, Kastrati A, Schühlen H, Blasini R, Hadamitzky M, Walter H, Zitzmann-Roth EM, Richardt G, Alt E, Schmitt C, Ulm K. A randomized comparison of antiplatelet and anticoagulant therapy after the placement of coronary-artery stents. *N Engl J Med* 1996; **334**: 1084-1089 [PMID: 8598866 DOI: 10.1056/NEJM199604253341702]

2 **Rao SV**, O'Grady K, Pieper KS, Granger CB, Newby LK, Van de Werf F, Mahaffey KW, Califf RM, Harrington RA. Impact of bleeding severity on clinical outcomes among patients with acute coronary syndromes. *Am J Cardiol* 2005; **96**: 1200-1206 [PMID: 16253582 DOI: 10.1016/j.amjcard.2005.06.056]

3 **Leon MB**, Baim DS, Popma JJ, Gordon PC, Cutlip DE, Ho KK, Giambartolomei A, Diver DJ, Lasorda DM, Williams DO, Pocock SJ, Kuntz RE. A clinical trial comparing three antithrombotic-drug regimens after coronary-artery stenting. Stent Anticoagulation Restenosis Study Investigators. *N Engl J Med* 1998; **339**: 1665-1671 [PMID: 9834303 DOI: 10.1056/NEJM199812033392303]

4 **Bertrand ME**, Legrand V, Boland J, Fleck E, Bonnier J, Emmanuelson H, Vrolix M, Missault L, Chierchia S, Casaccia M, Niccoli L, Oto A, White C, Webb-Peploe M, Van Belle E, McFadden EP. Randomized multicenter comparison of conventional anticoagulation versus antiplatelet therapy in unplanned and elective coronary stenting. The full anticoagulation versus aspirin and ticlopidine (fantastic) study. *Circulation* 1998; **98**: 1597-1603 [PMID: 9778323]

5 **Hagihara K**, Kazui M, Kurihara A, Yoshiike M, Honda K, Okazaki O, Farid NA, Ikeda T. A possible mechanism for the differences in efficiency and variability of active metabolite formation from thienopyridine antiplatelet agents, prasugrel and clopidogrel. *Drug Metab Dispos* 2009; **37**: 2145-2152 [PMID: 19704027 DOI: 10.1124/dmd.109.028498]

6 **Kazui M**, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O, Ikeda T, Kurihara A. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. *Drug Metab Dispos* 2010; **38**: 92-99 [PMID: 19812348 DOI: 10.1124/dmd.109.029132]

7 **Pereillo JM**, Maftouh M, Andrieu A, Uzabiaga MF, Fedeli O, Savi P, Pascal M, Herbert JM, Maffrand JP, Picard C. Structure and stereochemistry of the active metabolite of clopidogrel. *Drug Metab Dispos* 2002; **30**: 1288-1295 [PMID: 12386137]

8 **Yusuf S**, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med* 2001; **345**: 494-502 [PMID: 11519503 DOI: 10.1056/NEJMoa010746]

9 **Mehta SR**, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, Malmberg K, Rupprecht H, Zhao F, Chrolavicius S, Copland I, Fox KA. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. *Lancet* 2001; **358**: 527-533 [PMID: 11520521 DOI: 10.1016/S0140-6736(01)05701-4]

10 **Järemo P**, Lindahl TL, Fransson SG, Richter A. Individual variations of platelet inhibition after loading doses of clopidogrel. *J Intern Med* 2002; **252**: 233-238 [PMID: 12270003 DOI: 10.1046/j.1365-2796.2002.01027.x]

11 **Bonello L**, Tantry US, Marcucci R, Blindt R, Angiolillo DJ, Becker R, Bhatt DL, Cattaneo M, Collet JP, Cuisset T, Gachet

C, Montalescot G, Jennings LK, Kereiakes D, Sibbing D, Trenk D, Van Werkum JW, Paganelli F, Price MJ, Waksman R, Gurbel PA. Consensus and future directions on the definition of high on-treatment platelet reactivity to adenosine diphosphate. *J Am Coll Cardiol* 2010; **56**: 919-933 [PMID: 20828644 DOI: 10.1016/j.jacc.2010.04.047]

12 **Barragan P**, Bouvier JL, Roquebert PO, Macaluso G, Commeau P, Comet B, Lafont A, Camoin L, Walter U, Eigenthaler M. Resistance to thienopyridines: clinical detection of coronary stent thrombosis by monitoring of vasodilator-stimulated phosphoprotein phosphorylation. *Catheter Cardiovasc Interv* 2003; **59**: 295-302 [PMID: 12822144 DOI: 10.1002/ccd.10497]

13 **Taubert D**, von Beckerath N, Grimberg G, Lazar A, Jung N, Goeser T, Kastrati A, Schömig A, Schömig E. Impact of P-glycoprotein on clopidogrel absorption. *Clin Pharmacol Ther* 2006; **80**: 486-501 [PMID: 17112805 DOI: 10.1016/j.clpt.2006.07.007]

14 **Mega JL**, Close SL, Wiviott SD, Shen L, Walker JR, Simon T, Antman EM, Braunwald E, Sabatine MS. Genetic variants in ABCB1 and CYP2C19 and cardiovascular outcomes after treatment with clopidogrel and prasugrel in the TRITON-TIMI 38 trial: a pharmacogenetic analysis. *Lancet* 2010; **376**: 1312-1319 [PMID: 20801494 DOI: 10.1016/S0140-6736(10)61273-1]

15 **Hulot JS**, Bura A, Villard E, Azizi M, Remones V, Goyenvalle C, Aiach M, Lechat P, Gaussem P. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood* 2006; **108**: 2244-2247 [PMID: 16772608 DOI: 10.1182/blood-2006-04-013052]

16 **Brandt JT**, Close SL, Iturria SJ, Payne CD, Farid NA, Ernest CS, Lachno DR, Salazar D, Winters KJ. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. *J Thromb Haemost* 2007; **5**: 2429-2436 [PMID: 17900275 DOI: 10.1111/j.1538-7836.2007.02775.x]

17 **Trenk D**, Hochholzer W, Fromm MF, Chialda LE, Pahl A, Valina CM, Stratz C, Schmiebusch P, Bestehorn HP, Büttner HJ, Neumann FJ. Cytochrome P450 2C19 681G > A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. *J Am Coll Cardiol* 2008; **51**: 1925-1934 [PMID: 18482659 DOI: 10.1016/j.jacc.2007.12.056]

18 **Mega JL**, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias W, Braunwald E, Sabatine MS. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009; **360**: 354-362 [PMID: 19106084 DOI: 10.1056/NEJMoa0809171]

19 **Lau WC**, Waskell LA, Watkins PB, Neer CJ, Horowitz K, Hopp AS, Tait AR, Carville DG, Guyer KE, Bates ER. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug-drug interaction. *Circulation* 2003; **107**: 32-37 [PMID: 12515739 DOI: 10.1161/01.CIR.0000047060.60595.CC]

20 **Gilard M**, Arnaud B, Cornily JC, Le Gal G, Lacut K, Le Calvez G, Mansourati J, Mottier D, Abgrall JF, Boschat J. Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: the randomized, double-blind OCLA (Omeprazole CLopidogrel Aspirin) study. *J Am Coll Cardiol* 2008; **51**: 256-260 [PMID: 18206732 DOI: 10.1016/j.jacc.2007.06.064]

21 **Angiolillo DJ**, Fernandez-Ortiz A, Bernardo E, Ramírez C, Sabaté M, Jimenez-Quevedo P, Hernández R, Moreno R, Escaned J, Alfonso F, Bañuelos C, Costa MA, Bass TA, Macaya C. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. *Diabetes* 2005; **54**: 2430-2435 [PMID: 16046311 DOI: 10.2337/diabetes.54.8.2430]

22 **Bonello-Palot N**, Armero S, Paganelli F, Mancini J, De Labriolle A, Bonello C, Lévy N, Maillard L, Barragan P, Dignat-George F, Camoin-Jau L, Bonello L. Relation of body mass index to high on-treatment platelet reactivity and of failed clopidogrel dose adjustment according to platelet reactivity monitoring in patients undergoing percutaneous coronary intervention. *Am J Cardiol* 2009; **104**: 1511-1515 [PMID: 19932784 DOI: 10.1016/j.amjcard.2009.07.015]

23 **Sibbing D**, von Beckerath O, Schömig A, Kastrati A, von Beckerath N. Platelet function in clopidogrel-treated patients with acute coronary syndrome. *Blood Coagul Fibrinolysis* 2007; **18**: 335-339 [PMID: 17473574 DOI: 10.1097/MBC.0b013e3280d21aed]

24 **Guthikonda S**, Alviar CL, Vaduganathan M, Arikan M, Tellez A, DeLao T, Granada JF, Dong JF, Kleiman NS, Lev EI. Role of reticulated platelets and platelet size heterogeneity on platelet activity after dual antiplatelet therapy with aspirin and clopidogrel in patients with stable coronary artery disease. *J Am Coll Cardiol* 2008; **52**: 743-749 [PMID: 18718422 DOI: 10.1016/j.jacc.2008.05.031]

25 **Farid NA**, Smith RL, Gillespie TA, Rash TJ, Blair PE, Kurihara A, Goldberg MJ. The disposition of prasugrel, a novel thienopyridine, in humans. *Drug Metab Dispos* 2007; **35**: 1096-1104 [PMID: 17403916 DOI: 10.1124/dmd.106.014522]

26 **Rehmel JL**, Eckstein JA, Farid NA, Heim JB, Kasper SC, Kurihara A, Wrighton SA, Ring BJ. Interactions of two major metabolites of prasugrel, a thienopyridine antiplatelet agent, with the cytochromes P450. *Drug Metab Dispos* 2006; **34**: 600-607 [PMID: 16415119 DOI: 10.1124/dmd.105.007989]

27 **Sugidachi A**, Ogawa T, Kurihara A, Hagihara K, Jakubowski JA, Hashimoto M, Niitsu Y, Asai F. The greater in vivo antiplatelet effects of prasugrel as compared to clopidogrel reflect more efficient generation of its active metabolite with similar antiplatelet activity to that of clopidogrel's active metabolite. *J Thromb Haemost* 2007; **5**: 1545-1551 [PMID: 17456192 DOI: 10.1111/j.1538-7836.2007.02598.x]

28 **Wiviott SD**, Trenk D, Frelinger AL, O'Donoghue M, Neumann FJ, Michelson AD, Angiolillo DJ, Hod H, Montalescot G, Miller DL, Jakubowski JA, Cairns R, Murphy SA, McCabe CH, Antman EM, Braunwald E. Prasugrel compared with high loading- and maintenance-dose clopidogrel in patients with planned percutaneous coronary intervention: the Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation-Thrombolysis in Myocardial Infarction 44 trial. *Circulation* 2007; **116**: 2923-2932 [PMID: 18056526 DOI: 10.1161/CIRCULATIONAHA.107.740324]

29 **Wiviott SD**, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, Neumann FJ, Ardissino D, De Servi S, Murphy SA, Riesmeyer J, Weerakkody G, Gibson CM, Antman EM. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2007; **357**: 2001-2015 [PMID: 17982182 DOI: 10.1056/NEJMoa0706482]

30 **Roe MT**, Armstrong PW, Fox KA, White HD, Prabhakaran D, Goodman SG, Cornel JH, Bhatt DL, Clemmensen P, Martinez F, Ardissino D, Nicolau JC, Boden WE, Gurbel PA, Ruzyllo W, Dalby AJ, McGuire DK, Leiva-Pons JL, Parkhomenko A, Gottlieb S, Topacio GO, Hamm C, Pavlides G, Goudev AR, Oto A, Tseng CD, Merkely B, Gasparovic V, Corbalan R, Cinteză M, McLendon RC, Winters KJ, Brown EB, Lokhnygina Y, Aylward PE, Huber K, Hochman JS, Ohman EM. Prasugrel versus clopidogrel for acute coronary syndromes without revascularization. *N Engl J Med* 2012; **367**: 1297-1309 [PMID: 22920930 DOI: 10.1056/NEJMoa1205512]

31 **Montalescot G**, Bolognese L, Dudek D, Goldstein P, Hamm C, Tanguay JF, ten Berg JM, Miller DL, Costigan TM, Goedicke J, Silvain J, Angioli P, Legutko J, Niethammer M, Motovska Z, Jakubowski JA, Cayla G, Visconti LO, Vicaut E, Widimsky P. Pretreatment with prasugrel in non-ST-segment elevation acute coronary syndromes. *N Engl J Med* 2013; **369**: 999-1010 [PMID: 23991622 DOI: 10.1056/NEJMoa1308075]

32 **Teng R**, Oliver S, Hayes MA, Butler K. Absorption, distribution, metabolism, and excretion of ticagrelor in healthy subjects. *Drug Metab Dispos* 2010; **38**: 1514-1521 [PMID: 20551239 DOI: 10.1124/dmd.110.032250]

33 **Gurbel PA**, Bliden KP, Butler K, Tantry US, Gesheff T, Wei C, Teng R, Antonino MJ, Patil SB, Karunakaran A, Kereiakes DJ, Parris C, Purdy D, Wilson V, Ledley GS, Storey RF. Randomized double-blind assessment of the ONSET and OFFSET of the antiplatelet effects of ticagrelor versus clopidogrel in patients with stable coronary artery disease: the ONSET/OFFSET study. *Circulation* 2009; **120**: 2577-2585 [PMID: 19923168 DOI: 10.1161/CIRCULATIONAHA.109.912550]

34 **Wallentin L**, Becker RC, Budaj A, Cannon CP, Emanuelsson H,

Held C, Horrow J, Husted S, James S, Katus H, Mahaffey KW, Scirica BM, Skene A, Steg PG, Storey RF, Harrington RA, Freij A, Thorsén M. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2009; **361**: 1045-1057 [PMID: 19717846 DOI: 10.1056/NEJMoa0904327]

35 **Montalescot G**, van 't Hof AW, Lapostolle F, Silvain J, Lassen JF, Bolognese L, Cantor WJ, Cequier A, Chettibi M, Goodman SG, Hammett CJ, Huber K, Janzon M, Merkely B, Storey RF, Zeymer U, Stibbe O, Ecollan P, Heutz WM, Swahn E, Collet JP, Willems FF, Baradat C, Licour M, Tsatsaris A, Vicaut E, Hamm CW. Prehospital ticagrelor in ST-segment elevation myocardial infarction. *N Engl J Med* 2014; **371**: 1016-1027 [PMID: 25175921 DOI: 10.1056/NEJMoa1407024]

36 **Storey RF**, Sanderson HM, White AE, May JA, Cameron KE, Heptinstall S. The central role of the P(2T) receptor in amplification of human platelet activation, aggregation, secretion and procoagulant activity. *Br J Haematol* 2000; **110**: 925-934 [PMID: 11054084]

37 **Harrington RA**, Stone GW, McNulty S, White HD, Lincoff AM, Gibson CM, Pollack CV, Montalescot G, Mahaffey KW, Kleiman NS, Goodman SG, Amine M, Angiolillo DJ, Becker RC, Chew DP, French WJ, Leisch F, Parikh KH, Skerjanec S, Bhatt DL. Platelet inhibition with cangrelor in patients undergoing PCI. *N Engl J Med* 2009; **361**: 2318-2329 [PMID: 19915221 DOI: 10.1056/NEJMoa0908628]

38 **Bhatt DL**, Lincoff AM, Gibson CM, Stone GW, McNulty S, Montalescot G, Kleiman NS, Goodman SG, White HD, Mahaffey KW, Pollack CV, Manoukian SV, Widimsky P, Chew DP, Cura F, Manukov I, Tousek F, Jafar MZ, Arneja J, Skerjanec S, Harrington RA. Intravenous platelet blockade with cangrelor during PCI. *N Engl J Med* 2009; **361**: 2330-2341 [PMID: 19915222 DOI: 10.1056/NEJMoa0908629]

39 **Bhatt DL**, Stone GW, Mahaffey KW, Gibson CM, Steg PG, Hamm CW, Price MJ, Leonardi S, Gallup D, Bramucci E, Radke PW, Widimský P, Tousek F, Tauth J, Spriggs D, McLaurin BT, Angiolillo DJ, Généreux P, Liu T, Prats J, Todd M, Skerjanec S, White HD, Harrington RA. Effect of platelet inhibition with cangrelor during PCI on ischemic events. *N Engl J Med* 2013; **368**: 1303-1313 [PMID: 23473369 DOI: 10.1056/NEJMoa1300815]

40 **Angiolillo DJ**, Firstenberg MS, Price MJ, Tummala PE, Hutyra M, Welsby IJ, Voeltz MD, Chandna H, Ramaiah C, Brtko M, Cannon L, Dyke C, Liu T, Montalescot G, Manoukian SV, Prats J, Topol EJ. Bridging antiplatelet therapy with cangrelor in patients undergoing cardiac surgery: a randomized controlled trial. *JAMA* 2012; **307**: 265-274 [PMID: 22253393 DOI: 10.1001/jama.2011.2002]

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Mitochondrial vasculopathy

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Abstract

Mitochondrial disorders (MIDs) are usually multisystem disorders (mitochondrial multiorgan disorder syndrome) either on from onset or starting at a point during the disease course. Most frequently affected tissues are those with a high oxygen demand such as the central nervous system, the muscle, endocrine glands, or the myocardium. Recently, it has been shown that rarely also the arteries may be affected (mitochondrial arteriopathy). This review focuses on the type, diagnosis, and treatment of mitochondrial vasculopathy in MID patients. A literature search using appropriate search terms was carried out. Mitochondrial vasculopathy manifests as either microangiopathy or macroangiopathy. Clinical manifestations of mitochondrial microangiopathy include leukoencephalopathy, migraine-like headache, stroke-like episodes, or peripheral retinopathy. Mitochondrial macroangiopathy manifests as atherosclerosis, ectasia of arteries, aneurysm formation, dissection, or spontaneous rupture of arteries. The diagnosis relies on the documentation and confirmation of the mitochondrial metabolic defect or the genetic cause after exclusion of non-MID causes. Treatment is not at variance compared to treatment of vasculopathy due to non-MID causes. Mitochondrial vasculopathy exists and manifests as micro- or macroangiopathy. Diagnosing mitochondrial vasculopathy is crucial since appropriate treatment may prevent from severe complications.

Key words: Mitochondrial disorder; Multisystem; MtDNA; Phenotype; Vasculopathy; Arteriopathy; Angiopathy; Genotype

Core tip: Recently, it has been shown that rarely also the arteries may be affected in mitochondrial disorders, known as mitochondrial vasculopathy. Mitochondrial vasculopathy manifests as either microangiopathy or macroangiopathy. Clinical manifestations of mitochondrial microangiopathy include leukoencephalopathy, migraine-like headache, stroke-like episodes, or peripheral retinopathy. Mitochondrial macroangiopathy manifests as atherosclerosis, ectasia of arteries, aneurysm formation, dissection, or spontaneous rupture of arteries. The diagnosis relies on the documentation and confirmation of the mitochondrial metabolic defect or the genetic cause after exclusion of non-mitochondrial causes. Treatment is not at variance compared to treatment of vasculopathy

due to non-mitochondrial causes.

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INTRODUCTION

It is well established that syndromic and non-syndromic mitochondrial disorders (MIDs) manifest as mitochondrial multiorgan disorder syndrome (MIMODS) in the majority of the cases, either since onset of the disease or starting later during the disease course[1,2]. Organs or tissues involved in MIMODS are numerous but some are more frequently affected than others and some are better recognised as sites of involvement than others. Among the frequently affected organs are the muscle, brain, eyes, ears, endocrine organs, and the heart. Less frequently involved are the liver, pancreas, intestines, kidneys, blood, and skin. Hardly known and appreciated as possibly affected organs in MIDs are the lung and the arteries. Here we summarise and discuss recent findings concerning the involvement of the arteries, arterioles, and capillaries in MIDs (mitochondrial vasculopathy). Mitochondrial vasculopathy may manifest in large arteries (macroangiopathy) or the small arteries (microangiopathy). Microangiopathy is defined as vasculopathy of the small arteries, arterioles, capillaries, or venules[3]. This review aims at summarising and discussing recent findings concerning mitochondrial vasculopathy.

SEARCH STRATEGY

Data for this review were identified by searches of MEDLINE, Current Contents, EMBASE, Web of Science, Web of Knowledge, LILACS, SCOPUS, and Google Scholar for references of relevant articles using the search terms "vasculopathy", "macroangiopathy", "microangiopathy", "aortic ectasia", "dissection", "rupture", "aneurysm", "megadolichobasilar artery", "migraine", "migraine-like headache", "microvascular", and "stroke-like episode" in combination with "mitochondrial", "mtDNA", "respiratory chain", "oxidative phosphorylation", and "cytopathy". Randomized (blinded or open label) clinical trials, longitudinal studies, case series, and case reports were considered. Abstracts and reports from meetings were not included. Only articles published in English, French, Spanish, or German between 1966 and 2015 were considered. Appropriate papers were studied and discussed for their suitability to be incorporated in this review. Fifty-nine papers were identified as suitable to be discussed in this review. According to these papers mitochondrial vasculopathy may be classified according to various criteria.

CLASSIFICATION OF MITOCHONDRIAL VASCULOPATHY

Mitochondrial vasculopathy may not only be classified as micro- or macro-angiopathy but also as primary or secondary. Primary mitochondrial vasculopathy is due to affection of cells constituting the vessels by the causative metabolic defect. Secondary mitochondrial vasculopathy is due to affection of vessels secondary to the development of diabetes, hyperlipidemia, or arterial hypertension due to affection of organs other than the arteries by the metabolic defect. Additionally, mitochondrial vasculopathy may go along with clinical manifestations or without and it may occur in a focal or generalised distribution. Mitochondrial macroangiopathy and microangiopathy present with various manifestations.

Microangiopathy

Microangiopathy manifests clinically or may remain subclinical. Clinical manifestations of mitochondrial microangiopathy include leukoencephalopathy, migraine-like headache, stroke-like episodes (SLEs), or peripheral retinopathy. Subclinical manifestations include morphological abnormalities in mitochondria of vascular smooth muscle cells (VSMCs), of pericytes, or of endothelial cells[4].

Leukoencephalopathy: Leukoencephalopathy is a frequent central nervous system (CNS) abnormality in syndromic as well as non-syndromic MIDs. It may be patchy or confluent. It is so far unknown if leukoencephalopathy is due to a vascular pathology or represents a primary metabolic defect of neurons or glial cells. Evidence for a vasculopathy comes from several observations. In a 13yo mitochondrial encephalomyopathy, lactic acidosis, and SLEs (MELAS) patient with leukoencephalopathy post-mortem studies revealed generalised cerebral microangiopathy[5]. In a post-mortem study of two other patients with MELAS-syndrome due to the mtDNA mutation m.3243A > G, of which one also presented with migraine, COX-negative VSMCs were most frequently found in the walls of leptomeningeal and cortical arteries over all cerebral regions[6].

Migraine-like headache: Migraine-like headache is a frequent manifestation of syndromic and non-syndromic MIDs. Best known is migraine-like headache as a manifestation of MELAS-syndrome[7] but has been also reported in adult Leigh-syndrome[8], Alpers-Huttenlocher disease[9], myoclonic epilepsy with ragged-red fibers (MERRF)-syndrome[10], mitochondrial recessive ataxia syndrome[11], chronic progressive external ophthalmoplegia (CPEO)[12], Leber's hereditary optic neuropathy (LHON)[13], cyclic vomiting syndrome[14], mitochondrial depletion syndrome[15], and non-syndromic MIDs due to POLG1 mutations[16]. Among patients

carrying the m.3243A > G mutation the prevalence of migraine was 58%[7]. In a study of two patients with MELAS-syndrome, of which on one also had migraine, COX-deficiency and heteroplasmy rates of the causative mutation were highest in cortical and leptomeningeal arteries[6]. Though the pathogenesis of migraine-like headache is poorly understood there are some studies indicating vasculopathy in these patients. Vascular pathology is characterised by episodic changes of the diameter of small cerebral arteries[17]. According to the vascular hypothesis of migraine it is assumed that initially there is vasoconstriction followed by vasodilation[17,18]. A further hypothesis suggests that activation of the calcitonin-related peptide gene is responsible for hyperperfusion and migraine[19]. Additional evidence for mitochondrial dysfunction in migraine derives from the finding that increased influx of calcium increases oxidative stress, that muscle biopsy of patients with migraine may show mitochondrial abnormalities, that mtDNA polymorphisms may be increased in migraine patients, and that riboflavin, coenzyme-Q, niacin, and carnitine, all agents used in the treatment of MIDs, exhibit a beneficial effect for migraine[20].

SLEs: SLEs are the hallmark of MELAS-syndrome but may occur in other syndromic or non-syndromic MIDs as well. They are clinically indistinguishable from ischemic strokes but may additionally manifest with seizures, headache, confusional state, or lactic acidosis[21]. The morphological correlate of a SLE is the stroke-like lesion, which is not confined to a vascular territory and most frequently located in the temporo-occipital region[22]. Concerning the MRI findings of stroke-like lesions, acute, subacute, and chronic alterations have to be differentiated. In the acute and subacute stage there may be cortical hyperintensity on diffusion weighted imaging (DWI) and hypointensity on apparent diffusion coefficient (ADC) maps (cytotoxic edema). The subcortical compound of a stroke-like lesion in this stage shows up as hyperintensity on DWI and hyperintensity on ADC in the cortical and subcortical compound (vasogenic edema)[23]. However, there are also studies showing hyperintensity on DWI and hyperintensity on ADC (vasogenic edema) in the subcortical and cortical grey matter. Fluid attenuated inversion recovery (FLAIR) sequences in the acute stage may show hyperintensity and MR angiography prominent dilatation of arteries and PWI consecutive hyperperfusion in the affected areas[24]. On the contrary, PWI studies in the acute stage in another study showed decreased cerebral blood flow as well as decreased cerebral blood volume[23,25]. Mean transit-time and time-to-peak are prolonged in lesional and non-lesional areas. The chronic stage of a stroke-like lesion may show up as hyperintensity on FLAIR sequences and as iso- or hyperintensity on ADC maps[22]. 99mTc-hexa-methyl-propyl-eneamine-oxime single-photon emission CT (HMPAO-SPECT) of a stroke-like-lesion in the chronic stage may show hyperperfusion or a mixture of hypo- and hyperperfusion[19,23,26].

Three main hypotheses have been raised to explain the phenomenon. First, SLEs result from mitochondrial vasculopathy caused by dysfunction of VSMCs of the small cerebral arteries leading to disruption of the blood brain barrier and consecutive vasogenic respectively cytotoxic edema and neuronal death[19,21,23]. Second, mitochondrial dysfunction may secondarily cause impaired cellular or mitochondrial metabolism resulting in decreased mitochondrial energy production causing neuronal damage or neuronal death[21]. Third, an initial seizure may cause oxidative stress resulting in secondary metabolic break-down. An argument for the seizure hypothesis is that SLEs are frequently associated with seizures and that appropriate antiepileptic treatment may be beneficial also for stroke-like-episodes[27].

Microangiopathy of retinal arteries: LHON is a syndromic MID with subacute onset visual impairment leading to permanent blindness. Two thirds of these patients present with microangiopathy of the retinal arteries, characterised by increased tortuosity and ectasias[28-31]. The same vascular abnormalities may be also found in non-manifesting carriers of the disease. However, the pathogenetic role of these vascular changes remains questionable since not all LHON patients develop retinal microangiopathy[28]. In rare cases, retinal microangiopathy may spontaneously regress[32].

Subclinical mitochondrial vasculopathy: Further evidence for microangiopathy in MIDs comes from a study of 3 patients with MERRF, CPEO, and migraine respectively, each carrying the m.3243A > G mutation[33]. 99mTc-HMPAO-SPECT in these three patients revealed asymmetric hypoperfusion in various cerebral regions with predominance in the temporo-occipital regions[33]. In another study of 13 MELAS patients reactivity of the median cerebral artery to hypo- or hyper-capnia was decreased on transcranial Doppler sonography and there was crossed diaschisis[34]. In a study of patients with Leigh-syndrome capillary shunting was documented by cerebral MRI[35]. In a recent study of 16 MID patients carrying the m.3243A > G mutation, the m.8344A > G mutation, or a POLG1 mutation respectively, multiple ischemic-like lesions were found in the cerebellar cortex bilaterally[36]. The findings were attributed to dysfunction of VSMCs and endothelial cells[36]. Dysfunction of endothelial cells and VSMCs was made responsible for a breakdown of the blood-brain-barrier, resulting in extravasation of plasma proteins and disruption of tight junctions of endothelial cells[36]. In a patient carrying the m.3243A > G mutation histological studies of the skin and muscle showed extra-cellular matrix mineralization in blood vessel walls[37]. There was also a correlation between SDH histochemical staining and the number of mitochondria on electron microscopy[37]. In a boy with non-syndromic MID neuropathological work-up of the brain revealed spongiform changes, swelling of endothelial cells, and increased number of mitochondria

with abnormal cristae formation in pericytes and VSMCs[4]. In a girl with MELAS-syndrome generalised microangiopathy with reduced COX-activity was found in the cerebrum, myocardium, and skeletal muscle[38]. In a histopathological study of MELAS patients COX-deficiency and heteroplasmy rates were highest in cortical and leptomeningeal arteries[6]. In case muscular arteries are subclinically affected histological studies may show SDH hyperreactivity, also known as strongly-succinate dehydrogenase-reactive vessels (SSV)[26]. SSV may occur in MELAS[39-42], CPEO[43], MERRF[44], and MERRF/MELAS overlap syndrome[45]. SSV are usually normal for COX[44] but in 5 MERRF patients SSV were COX-negative[44]. Ultrastructural investigations may show cristae swelling and increased number of mitochondria in VSMCs and endothelial cells[25]. In patients with non-syndromic MID muscle biopsy showed vasculopathy with swollen endothelial cells and swollen and dysmorphic mitochondria in VSMCs and pericytes[4]. Muscle biopsy of MID patients may also show reduced NO bioactivity particularly in endothelial cells and VSMCs of these patients[46]. When studying chronic intestinal pseudo-obstruction in MNGIE patients it turned out that mtDNA depletion due to tyrosine phosphorylase gene mutations was also present in VSMCs and endothelial cells of small arteries within the gastrointestinal walls[47].

Macroangiopathy

Premature atherosclerosis: There is increasing evidence that abnormal premature primary atherosclerosis can be a prominent feature of MIDs. Though not systematically investigated, an increasing number of patients with mitochondrial atherosclerosis is reported indicating that premature atherosclerosis particularly in patients without classical risk factors for atherosclerosis occurs. In a 54yo male with recurrent hyper-CKemia, Leriche-syndrome developed in the absence of classical risk factors for atherosclerosis and despite regular extensive physical exercise in form of frequent bicycling[48]. In a MID patient carrying the m.617G > A mutation, recurrent embolic strokes originating from an internal carotid artery stenosis in the absence of classical risk factors for atherosclerosis was reported[19]. It was concluded that mtDNA mutations might be implicated in the development of macroangiopathy in MID patients[19].

Ectasia of arteries: Ectasia of arteries in MIDs has been described for the aorta and the cerebral arteries. Aortic root ectasia: Aortic root ectasia in MID patients has been first described by Brunetti-Pierri *et al*[49] in 2011 in 10 patients with non-syndromic MIDs. These ten patients had an increased Z-score of the aortic root width, which is zero per definition in controls. One of these patients was a female and ten were males, aged 0.5 to 11.5 years[49]. A further case with non-syndromic MID and aortic root ectasia has been recently recognised. In a 84yo female with suspected non-syndromic MID, ectasia of the aortic root was diagnosed on X-ray of the lungs and confirmed by CT of the aorta. Most likely, aortic root ectasia is more frequent among MID patients than so far appreciated. However, except for the study by Brunetti-Pierri *et al*[49] no further systematic investigations regarding this issue have been conducted. It is unknown if aortic root ectasia in MIDs is associated with an increased risk of aortic dissection type A. It is also unknown if MID patients with aortic root ectasia have a worse prognosis compared to MID patients without. Ectasia of cerebral arteries: Ectasia of arteries in MIDs has not only been reported for the aorta but in a single patient also for the basilary artery[50]. In a 70yo female with suspected MID, ectasia of the basilary artery has been demonstrated[50]. Originally, the patient was admitted for an ischemic stroke in the posterior leg of the left internal capsule. Features suggesting MID in this patient included leukoencephalopathy, short stature, and hyperlipidemia[50]. Since megadolichobasilar arteries are not infrequent, these patients should undergo investigations for a MID if time of flight angiography on cerebral MRI or CT angiography show ectasia of the cerebral arteries. Additionally, patients with a MID should be investigated for ectatic cerebral arteries as a CNS manifestation of the disease. Pathogenetically, it can be suspected that there is impaired innervation of the arterioles and thus reduced tone of the vessel wall, that there is a decrease of collagen fibers, or impairment of the VSMCs due to metabolic dysfunction. It is also conceivable that arterial ectasia is congenital without progression during the further course.

Aneurysm formation: Cerebral aneurysms are the most frequent cause of subarachnoid bleeding with often poor or fatal outcome[51]. Particularly, in cases with hereditary subarachnoid bleeding with maternal trait of inheritance a MID should be suspected and affected patients investigated appropriately. Also in cases of accidental detection of a cerebral aneurysm, which is the most frequent mode how cerebral aneurysms are diagnosed, the family history is of great importance and in case there are indications for a MID in one of the family members, appropriate diagnostic work-up should be initiated also in other family members. Since cerebral aneurysms may occur in a familial distribution it appears justified not only to investigate MID patients for cerebral aneurysms but also their affected and non-affected relatives. A pseudoaneurysm of the right internal carotid artery was found in a 47yo female with MELAS-syndrome[52].

Dissection: Spontaneous dissection of the carotid artery is a rare manifestation of a MID and has been reported only in five patients thus far[37,52,53]. In a patient carrying the mtDNA mutation m.3243A > G spontaneous dissection of the internal carotid artery and of the vertebral arteries was reported[37]. Skin and muscle biopsy in this patient revealed ragged-red fibers (RRFs), regional variability of succinate-dehydrogenase (SDH) histochemical reactivity, morphologically abnormal mitochondria, and accumulations of mitochondria[37].

Similar mitochondrial abnormalities and extracellular matrix mineralisation were found in arterial walls[37]. In three further patients with suspected MID, spontaneous dissection of the carotid artery and the posterior cerebral artery have been described[53]. Muscle biopsy in these patients revealed RRFs, SDH hyporeactivity, and COX-negative fibers[53]. These abnormalities were made responsible for the development of arteriopathy with dissection. Possibly, more MID patients have experienced arterial dissection but were either not reported or a causal relation was not assumed. Neurosurgeons, vascular surgeons, and neurologists must be aware of MIDs as the cause of carotid artery dissection and each patient with carotid artery dissection but without an evident cause should be investigated for a MID as well. Treatment is not at variance from that of dissection due to non-mitochondrial causes. The fifth patients is a 47yo female with mitochondrial myopathy due to the m.3243A > G mutation who presented a right carotid artery dissection with consecutive ischemic stroke in the right median cerebral artery territory[52].

Spontaneous rupture of arteries: In a 15yo girl with MELAS-syndrome due to the m.3243A > G mutation spontaneous rupture of the aorta during insertion of a gastrostomy has been reported[54]. Rupture of the aorta was attributed to affection of the aortic wall by the metabolic defect since post-mortem histological examination had revealed disorganised layers of VSMCs, disrupted elastic layers, and decreased COX staining of VSMCs of the vasa vasorum of the aorta[54]. Additionally, PCR and RFLP revealed a mutation load of 40% in blood lymphocytes but 85% in arteries[54]. Interestingly, the family history was positive for arteriopathy since the mother of this girl had died from rupture of a major artery during angiography[54]. Unfortunately, it was not mentioned if the deceased mother also suffered from a MID or not and if she manifested in organs other than the arteries[54].

Vascular malformations: In a single patient with LHON due to the mtDNA mutation m.11778G > A in the *ND4* gene, conventional cerebral angiography after right thalamic bleeding at age 9yo revealed an arteriovenous malformation with feeders from the posterior thalamo-perforat artery[55].

Reduced flow-mediated vasodilation: Flow-mediated vasodilation (FMD) is defined as change in diameter of an artery as assessed by high-resolution ultrasound in response to the release of an inflated cuff proximal to the measurement[26]. In a study of 35 patients with MELAS-syndrome the FMD was generally reduced[26]. In a study of 12 patients with a MID the FMD was reduced compared to controls[56].

DIAGNOSIS

Diagnosing mitochondrial vasculopathy is not at variance from non-mitochondrial vasculopathy. The diagnosis relies on the documentation and confirmation of the mitochondrial metabolic defect or the genetic cause and the exclusion of non-MID causes. In case of SLEs it is advisable to additionally carry out EEG recordings.

TREATMENT

Treatment of mitochondrial vasculopathy is not at variance from treatment of non-mitochondrial vasculopathy but patients with SLE may profit from L-arginine, vitamins, and coenzyme-Q[57]. Administration of co-factors may be also beneficial for MID patients with migraine-like headache. L-arginine may improve the FMD on a long-term basis[26].

DISCUSSION AND CONCLUSION

Diagnosing mitochondrial vasculopathy is important since it has several clinical implications. First, recognition of vasculopathy of undetermined cause may lead to the diagnosis of a MID. Diagnosing MIDs is important since many MIDs are frequently non-recognised for years or under-diagnosed. Mitochondrial vasculopathy most obviously indicating a MID as the underlying pathology is a stroke-like lesion. This is evident since stroke-like lesions are hallmarks of some MIDs and do not occur in disorders other than MIDs. Mitochondrial vasculopathy second most frequently indicative of a MID as an underlying cause is migraine[7]. Migraine is a frequent phenotypic feature of several syndromic and non-syndromic MIDs[7]. It may present not always as classical migraine why it is often termed migraine-like headache. Recent studies have shown that certain mtDNA polymorphisms are increased in patients with migraine[58]. There is also a mild bias towards a maternal transmission of migraine[59]. This is why patients with migraine should be suspected to have a MID as the underlying cause, as long as other possible causes have not been definitively excluded. Second, mitochondrial vasculopathy should be included as a phenotypic manifestation of syndromic or non-syndromic MIDs. Diagnosing MIDs should urge those manging MID patients to look for mitochondrial vasculopathy in individual patients and to initiate measures of treatment and prevention. Third, patients with MIDs should be systematically investigated for mitochondrial vasculopathy. This is important since MID patients are not investigated for concomitant mitochondrial vascular disease unless it is the dominant feature or leads to the diagnosis of LHON. Systematic investigations of MID patients for mitochondrial vasculopathy are important since early diagnosis may prevent severe complications. Systematic search for mitochondrial vasculopathy may contribute to assessing the prevalence of mitochondrial vasculopathy.

REFERENCES

1 **Finsterer J**, Bastovansky A. Multiorgan disorder syndrome (MODS) in an octagenarian suggests mitochondrial disorder. *Rev*

Med Chil 2015; **143**: 1210-1214 [PMID: 26530206 DOI: 10.4067/S0034-98872015000900016]
2 **Finsterer J**, Frank M. Diagnosing Mitochondrial Disorder without Sophisticated Means. *Acta Med Iran* 2015; **53**: 659-662 [PMID: 26615382]
3 **O'Riely V**. Microangiopathy. New York, NY: Hayle Medical, 2015
4 **Coquet M**, Fontan D, Vital C, Tudesq N, Baronnet R. [Muscle and brain biopsy in a case of mitochondrial encephalomyopathy. Demonstration of a mitochondrial vasculopathy]. *Ann Pathol* 1990; **10**: 181-186 [PMID: 2386601]
5 **Ohara S**, Ohama E, Takahashi H, Ikuta F, Nishizawa M, Tanaka K, Miyatake T. Alterations of oligodendrocytes and demyelination in the spinal cord of patients with mitochondrial encephalomyopathy. *J Neurol Sci* 1988; **86**: 19-29 [PMID: 3171595 DOI: 10.1016/0022-510X(88)90004-4]
6 **Betts J**, Jaros E, Perry RH, Schaefer AM, Taylor RW, Abdel-All Z, Lightowlers RN, Turnbull DM. Molecular neuropathology of MELAS: level of heteroplasmy in individual neurones and evidence of extensive vascular involvement. *Neuropathol Appl Neurobiol* 2006; **32**: 359-373 [PMID: 16866982 DOI: 10.1111/j.1365-2990.2006.00731.x]
7 **Guo S**, Esserlind AL, Andersson Z, Frederiksen AL, Olesen J, Vissing J, Ashina M. Prevalence of migraine in persons with the 3243A& gt; G mutation in mitochondrial DNA. *Eur J Neurol* 2016; **23**: 175-181 [PMID: 26435168 DOI: 10.1111/ene.12832]
8 **Dermaut B**, Seneca S, Dom L, Smets K, Ceulemans L, Smet J, De Paepe B, Tousseyn S, Weckhuysen S, Gewillig M, Pals P, Parizel P, De Bleecker JL, Boon P, De Meirleir L, De Jonghe P, Van Coster R, Van Paesschen W, Santens P. Progressive myoclonic epilepsy as an adult-onset manifestation of Leigh syndrome due to m.14487T& gt; C. *J Neurol Neurosurg Psychiatry* 2010; **81**: 90-93 [PMID: 20019223 DOI: 10.1136/jnnp.2008.157354]
9 **Uusimaa J**, Hinttala R, Rantala H, Päivärinta M, Herva R, Röyttä M, Soini H, Moilanen JS, Remes AM, Hassinen IE, Majamaa K. Homozygous W748S mutation in the POLG1 gene in patients with juvenile-onset Alpers syndrome and status epilepticus. *Epilepsia* 2008; **49**: 1038-1045 [PMID: 18294203 DOI: 10.1111/j.1528-1167.2008.01544.x]
10 **Sano M**, Ozawa M, Shiota S, Momose Y, Uchigata M, Goto Y. The T-C(8356) mitochondrial DNA mutation in a Japanese family. *J Neurol* 1996; **243**: 441-444 [PMID: 8803815 DOI: 10.1007/BF00900496]
11 **Tam EW**, Feigenbaum A, Addis JB, Blaser S, Mackay N, Al-Dosary M, Taylor RW, Ackerley C, Cameron JM, Robinson BH. A novel mitochondrial DNA mutation in COX1 leads to strokes, seizures, and lactic acidosis. *Neuropediatrics* 2008; **39**: 328-334 [PMID: 19568996 DOI: 10.1055/s-0029-1202287]
12 **Pfeffer G**, Sirrs S, Wade NK, Mezei MM. Multisystem disorder in late-onset chronic progressive external ophthalmoplegia. *Can J Neurol Sci* 2011; **38**: 119-123 [PMID: 21156440 DOI: 10.1017/S031716710001115X]
13 **Cupini LM**, Massa R, Floris R, Manenti G, Martini B, Tessa A, Nappi G, Bernardi G, Santorelli FM. Migraine-like disorder segregating with mtDNA 14484 Leber hereditary optic neuropathy mutation. *Neurology* 2003; **60**: 717-719 [PMID: 12601121 DOI: 10.1212/01.WNL.0000048662.77572.FB]
14 **Martinez-Esteve Melnikova A**, Schäppi MG, Korff C. Riboflavin in cyclic vomiting syndrome: efficacy in three children. *Eur J Pediatr* 2016; **175**: 131-135 [PMID: 26226892 DOI: 10.1007/s00431-015-2597-2]
15 **Finsterer J**, G Kovacs G, Ahting U. Adult mitochondrial DNA depletion syndrome with mild manifestations. *Neurol Int* 2013; **5**: 28-30 [PMID: 23888212 DOI: 10.4081/ni.2013.e9]
16 **Winterthun S**, Ferrari G, He L, Taylor RW, Zeviani M, Turnbull DM, Engelsen BA, Moen G, Bindoff LA. Autosomal recessive mitochondrial ataxic syndrome due to mitochondrial polymerase gamma mutations. *Neurology* 2005; **64**: 1204-1208 [PMID: 15824347 DOI: 10.1212/01.WNL.0000156516.77696.5A]
17 **Yoshida T**, Ouchi A, Miura D, Shimoji K, Kinjo K, Sueyoshi T, Jonosono M, Rajput V. MELAS and reversible vasoconstriction of the major cerebral arteries. *Intern Med* 2013; **52**: 1389-1392 [PMID: 23774553 DOI: 10.2169/internalmedicine.52.0188]
18 **Sacco S**, Ripa P, Grassi D, Pistoia F, Ornello R, Carolei A, Kurth T. Peripheral vascular dysfunction in migraine: a review. *J Headache Pain* 2013; **14**: 80 [PMID: 24083826 DOI: 10.1186/1129-2377-14-80]
19 **Iizuka T**, Goto Y, Miyakawa S, Sato M, Wang Z, Suzuki K, Hamada J, Kurata A, Sakai F. Progressive carotid artery stenosis with a novel tRNA phenylalanine mitochondrial DNA mutation. *J Neurol Sci* 2009; **278**: 35-40 [PMID: 19091329 DOI: 10.1016/j.jns.2008.11.016]
20 **Yorns WR**, Hardison HH. Mitochondrial dysfunction in migraine. *Semin Pediatr Neurol* 2013; **20**: 188-193 [PMID: 24331360 DOI: 10.1016/j.spen.2013.09.002]
21 **Lorenzoni PJ**, Werneck LC, Kay CS, Silvado CE, Scola RH. When should MELAS (Mitochondrial myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like episodes) be the diagnosis? *Arq Neuropsiquiatr* 2015; **73**: 959-967 [PMID: 26517220 DOI: 10.1590/0004-282X20150154]
22 **Finsterer J**. Stroke and Stroke-like Episodes in Muscle Disease. *Open Neurol J* 2012; **6**: 26-36 [PMID: 22715346 DOI: 10.2174/1874205X01206010026]
23 **Kim JH**, Lim MK, Jeon TY, Rha JH, Eo H, Yoo SY, Shu CH. Diffusion and perfusion characteristics of MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episode) in thirteen patients. *Korean J Radiol* 2011; **12**: 15-24 [PMID: 21228936 DOI: 10.3348/kjr.2011.12.1.15]
24 **Minobe S**, Matsuda A, Mitsuhashi T, Ishikawa M, Nishimura Y, Shibata K, Ito E, Goto Y, Nakaoka T, Sakura H. Vasodilatation of multiple cerebral arteries in early stage of stroke-like episode with MELAS. *J Clin Neurosci* 2015; **22**: 407-408 [PMID: 25128282 DOI: 10.1016/j.jocn.2014.05.021]
25 **Zhang ZQ**, Niu ST, Liang XH, Jian F, Wang Y. Vascular involvement in the pathogenesis of mitochondrial encephalomyopathies. *Neurol Res* 2010; **32**: 403-408 [PMID: 20483008 DOI: 10.1179/016164110X12670144526345]
26 **Koga Y**, Akita Y, Junko N, Yatsuga S, Povalko N, Fukiyama R, Ishii M, Matsuishi T. Endothelial dysfunction in MELAS improved by l-arginine supplementation. *Neurology* 2006; **66**: 1766-1769 [PMID: 16769961 DOI: 10.1212/01.wnl.0000220197.36849.1e]
27 **Finsterer J, Frank M**. MELAS or more. *Arq Neuropsiqiatr* 2016; Epub ahead of print
28 **Harding AE**, Riordan-Eva P, Govan GG. Mitochondrial DNA diseases: genotype and phenotype in Leber's hereditary optic neuropathy. *Muscle Nerve Suppl* 1995; **3**: S82-S84 [PMID: 7603533 DOI: 10.1002/mus.880181417]
29 **Luberichs J**, Leo-Kottler B, Besch D, Fauser S. A mutational hot spot in the mitochondrial ND6 gene in patients with Leber's hereditary optic neuropathy. *Graefes Arch Clin Exp Ophthalmol* 2002; **240**: 96-100 [PMID: 11931086 DOI: 10.1007/s00417-001-0423-1]
30 **Martin-Kleiner I**, Gabrilovac J, Bradvica M, Vidović T, Cerovski B, Fumić K, Boranić M. Leber's hereditary optic neuroretinopathy (LHON) associated with mitochondrial DNA point mutation G11778A in two Croatian families. *Coll Antropol* 2006; **30**: 171-174 [PMID: 16617593]
31 **Sadun F**, De Negri AM, Carelli V, Salomao SR, Berezovsky A, Andrade R, Moraes M, Passos A, Belfort R, da Rosa AB, Quiros P, Sadun AA. Ophthalmologic findings in a large pedigree of 11778/Haplogroup J Leber hereditary optic neuropathy. *Am J Ophthalmol* 2004; **137**: 271-277 [PMID: 14962416 DOI: 10.1016/j.ajo.2003.08.010]
32 **Sugisaka E**, Ohde H, Shinoda K, Mashima Y. Woman with atypical unilateral Leber's hereditary optic neuropathy with visual improvement. *Clin Experiment Ophthalmol* 2007; **35**: 868-870 [PMID: 18173420 DOI: 10.1111/j.1442-9071.2007.01628.x]
33 **Thajeb P**, Wu MC, Shih BF, Tzen CY, Chiang MF, Yuan RY. Brain single photon emission computed tomography in patients with A3243G mutation in mitochondrial DNA tRNA. *Ann N Y Acad Sci* 2005; **1042**: 48-54 [PMID: 15965044 DOI: 10.1196/annals.1338.005]
34 **Kodaka R**, Itagaki Y, Matsumoto M, Nagai T, Okada S. A

transcranial doppler ultrasonography study of cerebrovascular CO2 reactivity in mitochondrial encephalomyopathy. *Stroke* 1996; **27**: 1350-1353 [PMID: 8711801 DOI: 10.1161/01.STR.27.8.1350]

35 **Morin C**, Dubé J, Robinson BH, Lacroix J, Michaud J, De Braekeleer M, Geoffroy G, Lortie A, Blanchette C, Lambert MA, Mitchell GA. Stroke-like episodes in autosomal recessive cytochrome oxidase deficiency. *Ann Neurol* 1999; **45**: 389-392 [PMID: 10072055 DOI: 10.1002/1531-8249(199903)45:3<389::AID-ANA16>3.0.CO;2-B]

36 **Lax NZ**, Pienaar IS, Reeve AK, Hepplewhite PD, Jaros E, Taylor RW, Kalaria RN, Turnbull DM. Microangiopathy in the cerebellum of patients with mitochondrial DNA disease. *Brain* 2012; **135**: 1736-1750 [PMID: 22577219 DOI: 10.1093/brain/aws110]

37 **Sakharova AV**, Kalashnikova LA, Chaĭkovskaia RP, Mir-Kasimov MF, Nazarova MA, Pykhtina TN, Dobrynina LA, Patrusheva NL, Patrushev LI, Protskiĭ SV. [Morphological signs of mitochondrial cytopathy in skeletal muscles and micro-vessel walls in a patient with cerebral artery dissection associated with MELAS syndrome]. *Arkh Patol* 2012; **74**: 51-56 [PMID: 22880419]

38 **Müller-Höcker J**, Hübner G, Bise K, Förster C, Hauck S, Paetzke I, Pongratz D, Kadenbach B. Generalized mitochondrial microangiopathy and vascular cytochrome c oxidase deficiency. Occurrence in a case of MELAS syndrome with mitochondrial cardiomyopathy-myopathy and combined complex I/IV deficiency. *Arch Pathol Lab Med* 1993; **117**: 202-210 [PMID: 8381271]

39 **Shinde A**, Nakano S, Taguchi Y, Kagawa D, Akiguchi I. [A patient of MELAS with 3271 mutation with fatal outcome after alcohol intake]. *Rinsho Shinkeigaku* 2000; **40**: 561-565 [PMID: 11086393]

40 **Goto Y**, Horai S, Matsuoka T, Koga Y, Nihei K, Kobayashi M, Nonaka I. Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS): a correlative study of the clinical features and mitochondrial DNA mutation. *Neurology* 1992; **42**: 545-550 [PMID: 1549215 DOI: 10.1212/WNL.42.3.545]

41 **Ihara M**, Tanaka H, Yashiro M, Nishimura Y. [Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) with chronic renal failure: report of mother-child cases]. *Rinsho Shinkeigaku* 1996; **36**: 1069-1073 [PMID: 8976130]

42 **Mitsuoka T**, Kawarai T, Watanabe C, Katayama S, Nakamura S. [Comparison of clinical pictures of mitochondrial encephalomyopathy with tRNA(Leu(UUR)) mutation in 3243 with that in 3254]. *No To Shinkei* 1998; **50**: 1089-1092 [PMID: 9989353]

43 **Hasegawa H**, Matsuoka T, Goto Y, Nonaka I. [Vascular pathology in chronic progressive external ophthalmoplegia with ragged-red fibers]. *Rinsho Shinkeigaku* 1992; **32**: 155-160 [PMID: 1611773]

44 **Hasegawa H**, Matsuoka T, Goto Y, Nonaka I. Cytochrome c oxidase activity is deficient in blood vessels of patients with myoclonus epilepsy with ragged-red fibers. *Acta Neuropathol* 1993; **85**: 280-284 [PMID: 8384773 DOI: 10.1007/BF00227723]

45 **Yamazaki M**, Igarashi H, Hamamoto M, Miyazaki T, Nonaka I. [A case of mitochondrial encephalomyopathy with schizophrenic psychosis, dementia and neuroleptic malignant syndrome]. *Rinsho Shinkeigaku* 1991; **31**: 1219-1223 [PMID: 1813191]

46 **Vattemi G**, Mechref Y, Marini M, Tonin P, Minuz P, Grigoli L, Guglielmi V, Klouckova I, Chiamulera C, Meneguzzi A, Di Chio M, Tedesco V, Lovato L, Degan M, Arcaro G, Lechi A, Novotny MV, Tomelleri G. Increased protein nitration in mitochondrial diseases: evidence for vessel wall involvement. *Mol Cell Proteomics* 2011; **10**: M110.002964 [PMID: 21156839 DOI: 10.1074/mcp.M110.002964]

47 **Giordano C**, Sebastiani M, De Giorgio R, Travaglini C, Tancredi A, Valentino ML, Bellan M, Cossarizza A, Hirano M, d'Amati G, Carelli V. Gastrointestinal dysmotility in mitochondrial neurogastrointestinal encephalomyopathy is caused by mitochondrial DNA depletion. *Am J Pathol* 2008; **173**: 1120-1128 [PMID: 18787099 DOI: 10.2353/ajpath.2008.080252]

48 **Finsterer J**, Stöllberger C. Leriche-syndrome despite regular sport and non-compaction suggest neuromuscular disease. *Int J Cardiol* 2015; **191**: 15-17 [PMID: 25957931 DOI: 10.1016/j.ijcard.2015.04.279]

49 **Brunetti-Pierri N**, Pignatelli R, Fouladi N, Towbin JA, Belmont JW, Craigen WJ, Wong LJ, Jefferies JL, Scaglia F. Dilation of the aortic root in mitochondrial disease patients. *Mol Genet Metab* 2011; **103**: 167-170 [PMID: 21406331 DOI: 10.1016/j.ymgme.2011.02.007]

50 **Finsterer J**, Bastovansky A. Dilative Arteriopathy and Leucencephalopathy as Manifestations of a Neurometabolic Disease. *Open Neurol J* 2015; **9**: 28-31 [PMID: 26191091 DOI: 10.2174/1874205X01509010028]

51 **Beez T**, Steiger HJ, Hänggi D. Evolution of Management of Intracranial Aneurysms in Children: A Systematic Review of the Modern Literature. *J Child Neurol* 2016; **31**: 773-783 [PMID: 26516106]

52 **Ryther RC**, Cho-Park YA, Lee JW. Carotid dissection in mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes. *J Neurol* 2011; **258**: 912-914 [PMID: 21076841 DOI: 10.1007/s00415-010-5818-7]

53 **Kalashnikova LA**, Dobrynina LA, Sakharova AV, Chaĭkovskaia RP, Nazarova MA, Mir-Kasimov MF, Patrusheva NL, Patrushev LI, Konovalov RN, Protskiĭ SV. [The A3243G mitochondrial DNA mutation in cerebral artery dissections]. *Zh Nevrol Psikhiatr Im S S Korsakova* 2012; **112**: 84-89 [PMID: 22678682]

54 **Tay SH**, Nordli DR, Bonilla E, Null E, Monaco S, Hirano M, DiMauro S. Aortic rupture in mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes. *Arch Neurol* 2006; **63**: 281-283 [PMID: 16476819]

55 **Fujitake J**, Mizuta H, Fujii H, Ishikawa Y, Sasamoto K, Goto Y, Nonaka I, Tatsuoka Y. Leber's hereditary optic neuropathy with intracranial arteriovenous malformation: a case report. *Acta Neurol Belg* 2002; **102**: 82-86 [PMID: 12161905]

56 **Minuz P**, Fava C, Vattemi G, Arcaro G, Riccadonna M, Tonin P, Meneguzzi A, Degan M, Guglielmi V, Lechi A, Tomelleri G. Endothelial dysfunction and increased oxidative stress in mitochondrial diseases. *Clin Sci* (Lond) 2012; **122**: 289-297 [PMID: 21970465 DOI: 10.1042/CS20110199]

57 **El-Hattab AW**, Adesina AM, Jones J, Scaglia F. MELAS syndrome: Clinical manifestations, pathogenesis, and treatment options. *Mol Genet Metab* 2015; **116**: 4-12 [PMID: 26095523 DOI: 10.1016/j.ymgme.2015.06.004]

58 **Wang Q**, Ito M, Adams K, Li BU, Klopstock T, Maslim A, Higashimoto T, Herzog J, Boles RG. Mitochondrial DNA control region sequence variation in migraine headache and cyclic vomiting syndrome. *Am J Med Genet A* 2004; **131**: 50-58 [PMID: 15368478 DOI: 10.1002/ajmg.a.30323]

59 **Klopstock T**, May A, Seibel P, Papagiannuli E, Diener HC, Reichmann H. Mitochondrial DNA in migraine with aura. *Neurology* 1996; **46**: 1735-1738 [PMID: 8649580]

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ORIGINAL ARTICLE

Basic Study

Tenascin C upregulates interleukin-6 expression in human cardiac myofibroblasts *via* toll-like receptor 4

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Author contributions: Maqbool A performed the majority of the experiments and manuscript preparation; Spary EJ performed the statistical analysis; Manfield IW was involved in purification of the Tenascin C; Ruhmann M, Zuliani-Alvarez L and Midwood KS were equally involved with the synthesis and purification of the Tenascin C recombinants; Porter KE supplied the human cardiac fibroblasts; Drinkhill MJ generated the mouse infarct model; Gamboa-Esteves FO performed the histology, immunohistochemistry and image capture; Turner NA performed some of the experiments and was involved in experimental design and manuscript preparation.

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Institutional animal care and use committee statement: All procedures involving animals were carried out in accordance with the Home Office Animals (Scientific Procedures) Act 1986 and the University of Leeds Animal Welfare and Ethical Committee.

Conflict-of-interest statement: None.

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Abstract

AIM: To investigate the effect of Tenascin C (TNC) on the expression of pro-inflammatory cytokines and matrix

metalloproteinases in human cardiac myofibroblasts (CMF).

METHODS: CMF were isolated and cultured from patients undergoing coronary artery bypass grafting. Cultured cells were treated with either TNC (0.1 μmol/L, 24 h) or a recombinant protein corresponding to different domains of the TNC protein; fibrinogen-like globe (FBG) and fibronectin type Ⅲ-like repeats (TNⅢ 5-7) (both 1 μmol/L, 24 h). The expression of the pro-inflammatory cytokines; interleukin (IL)-6, IL-1β, TNFα and the matrix metalloproteinases; MMPs (MMP1, 2, 3, 9, 10, MT1-MMP) was assessed using real time RT-PCR and western blot analysis.

RESULTS: TNC increased both IL-6 and MMP3 (P < 0.01) mRNA levels in cultured human CMF but had no significant effect on the other markers studied. The increase in IL-6 mRNA expression was mirrored by an increase in protein secretion as assessed by enzyme-linked immunosorbant assay (P < 0.01). Treating CMF with the recombinant protein FBG increased IL-6 mRNA and protein (P < 0.01) whereas the recombinant protein TNⅢ 5-7 had no effect. Neither FBG nor TNⅢ 5-7 had any significant effect on MMP3 expression. The expression of toll-like receptor 4 (TLR4) in human CMF was confirmed by real time RT-PCR, western blot and immunohistochemistry. Pre-incubation of cells with TLR4 neutralising antisera attenuated the effect of both TNC and FBG on IL-6 mRNA and protein expression.

CONCLUSION: TNC up-regulates IL-6 expression in human CMF, an effect mediated through the FBG domain of TNC and *via* the TLR4 receptor.

Key words: Tenascin C; Matrix metalloproteinase; Toll-like receptor; Interleukin-6; Cardiac fibroblasts

Core tip: Tenascin C (TNC) is transiently expressed in cardiac tissue following acute myocardial infarction (MI) and MI patients with higher serum TNC levels have worse long term prognosis. This suggests that TNC is important in ventricular remodelling, although a functional role in this process is unclear. We report that TNC stimulates interleukin-6 synthesis in cardiac myofibroblasts, an effect mediated by toll-like receptor 4. As a growing body of evidence suggests that prolongation of the post-infarction inflammatory response results in worse remodelling and dysfunction this important observation may in part explain the mechanism by which TNC induces maladaptive ventricular remodelling following MI.

Maqbool A, Spary EJ, Manfield IW, Ruhmann M, Zuliani-Alvarez L, Gamboa-Esteves FO, Porter KE, Drinkhill MJ, Midwood KS, Turner NA. Tenascin C upregulates interleukin-6 expression in human cardiac myofibroblasts *via* toll-like receptor 4. *World J Cardiol* 2016; 8(5): 340-350 Available from: URL: http://www.wjgnet.com/1949-8462/full/v8/i5/340.htm DOI: http://dx.doi.org/10.4330/wjc.v8.i5.340

INTRODUCTION

A number of cardiac pathologies including acute myocardial infarction (MI), ischaemia/reperfusion (I/R) injury, hypertensive heart disease and myocarditis are associated with activation of pro-inflammatory mediators in the heart. Sustained expression of pro-inflammatory cytokines such as tumor necrosis factor-α (TNFα), interleukin (IL)-1 and IL-6 by infiltrating/resident inflammatory cells and cardiac fibroblasts is associated with increased matrix metalloproteinase (MMP) production, adverse cardiac remodelling leading to fibrosis, left ventricular (LV) dysfunction and heart failure[1]. The mechanisms that drive the inflammatory response in the heart are not fully understood.

The matricellular protein Tenascin C (TNC) is an extracellular glycoprotein that is highly expressed during embryonic development but is absent from healthy adult tissue. It is re-expressed in adult tissue during wound healing, inflammation and cancer invasion. In the adult myocardium TNC is up-regulated in pathological conditions that are closely associated with inflammation and extensive tissue remodelling such as MI[2], myocarditis[3] and dilated cardiomyopathy[4]. TNC is synthesised by interstitial fibroblasts and is up-regulated by various pro-inflammatory cytokines (*e.g.*, IL-1, IL-4, IL-13) and growth factors, as well as by oxidative and mechanical stress[5-11]. Evidence suggests that TNC may promote wound healing by recruiting cardiac myofibroblasts (CMF) during tissue repair[12]. However, it may also contribute to adverse ventricular remodelling as it can upregulate MMP production leading to excessive extracellular matrix (ECM) degradation. This in turn could weaken the adhesion of cardiomyocytes to the ECM, leading to cardiomyocyte slippage, LV dilation and reduction in contractile function[13]. Recent studies using synovial fibroblasts demonstrated that TNC was an endogenous activator of the toll-like receptor 4 (TLR4) pathway in the arthritic joint[14].

TLRs play a key role in driving inflammation and ECM turnover. They promote innate and adaptive immune responses including induction of pro-inflammatory cytokines and MMPs[15-18]. TLR4 has been identified on cardiac myocytes and TLR4 signalling is involved in the expression of cytokines in the myocardium[19-21]. Moreover, the TLR4 signalling pathway has been implicated in maladaptive ventricular remodelling[1] and in cardiac dysfunction after global I/R[22].

In the present study we investigated the effect of TNC on IL-6, IL-1β and MMP expression in a key cell type involved in the myocardial remodelling process, namely the human CMF[23,24]. In particular we investigated the interplay between TNC, TLR4 and the pro-inflammatory cytokine IL-6.

MATERIALS AND METHODS

Reagents

All cell culture reagents were purchased from Invitrogen (Paisley, Scotland, United Kingdom), except foetal calf serum (FCS) which was from Biosera (Ringmer, East Sussex, United Kingdom). Native human TNC was obtained from AbD Serotec (#8640-0502, Oxford, United Kingdom) and recombinant IL-1α from Life Technologies (Paisley, Scotland, United Kingdom). Lipopolysaccharide (LPS) was obtained from Sigma (Poole, United Kingdom).

Purification of human TNC

Purified human TNC protein (CC065, Millipore) from the human glioma cell line U251 was used in the *in vitro* experiments. Endotoxin levels were measured using the ToxinSensor Chromogenic LAL Endotoxin Assay Kit (Genscript). The TNC protein was taken through an endotoxin removal process using Detoxi-Gel Endotoxin Removal Columns (Thermoscientific) following the manufacturer's instructions. Commercial TNC, which was initially found to have an LPS concentration of approximately 15.8 pg/μg protein by LAL test, was column purified and found thereafter to be almost devoid of contamination (*i.e.*, < 0.1 pg/μg protein, which equates to < 3 pg/mL per reaction). The levels of LPS contamination of the TNC recombinant proteins [fibrinogen-like globe (FBG) and TNⅢ 5-7] was less than 10 pg/mL as described earlier[14]. These levels of contamination were more than 300-fold lower than that required (*i.e.*, 1 ng/mL) to stimulate IL-6 mRNA expression in human CMF. Nevertheless, polymyxin B was added in our experiments to block the biological effects of LPS. There was no evidence that polymyxin B alone could trigger IL-6 mRNA expression.

Recombinant TNC fragments

Recombinant TNC fragments corresponding to the FBG and fibronectin type Ⅲ-like repeats (TNⅢ 5-7) regions of the TNC protein were synthesised and purified as described previously[14].

Cell culture

Right atrial appendage biopsies from patients undergoing elective coronary artery bypass surgery at the Leeds General Infirmary were obtained following local ethical committee approval (reference number: 01/040) and informed patient consent. All investigations conformed to the principles outlined in the Declaration of Helsinki, 1997. Human cardiac fibroblasts were harvested, characterised and cultured as we have previously described[25,26]. Cells exhibit a myofibroblast phenotype as determined by positive staining for both α-smooth muscle actin and vimentin at passage 1 through to at least passage 5[26]. Experiments were performed on cells from passage 3-5 obtained from multiple donors. Cells were serum starved for 24 h before performing experiments in basal medium (DMEM) supplemented with 0.4% FCS and polymyxin B (50 μg/mL), an LPS neutralising agent, to ensure that any residual LPS in the TNC could not elicit a signal. Cells were treated with either IL-1α (10 ng/mL, 24 h), TNC (0.1 μmol/L, 24 h) or TNC recombinant fragments (FBG, TNIII 5-7, 1 μmol/L, 24 h). Concentrations and time point were based on preliminary dose response and time course experiments (data not shown). In TLR4 neutralising experiments, cells were pre-treated with TLR4 neutralising antibody (25 μg/mL, #AF1478, R and D Systems, Minneapolis, United States) for 1 h prior to TNC addition.

Quantitative RT-PCR

Cellular RNA was extracted from cells at the end of the incubation period and cDNA was prepared as described previously[27]. Real time RT-PCR was performed in duplicate using the Applied Biosystems 7500 Real-Time PCR System. Intron spanning primers and Taqman probes for human IL-1β (Hs00174097_m1), IL-6 (Hs00174131_m1), TNFα (Hs00174128_m1), MMP-1 (Hs00233958_m1), MMP-2 (Hs00234422_m1), MMP-3 (Hs00233962_m1), MMP-9 (Hs00234579_m1), MMP-10 (Hs00233987_m1), MT1-MMP (Hs00237119_m1), TLR2 (Hs01872448_s1) and TLR4 (Hs00152939_m1) were from Applied Biosystems (www.appliedbiosystems.com). Data are presented as a relative percentage of expression of the endogenous control GAPDH (Hs999-99905_m1 primers) using the formula $2^{-\Delta C_T} \times 100$ in which C_T is the cycle threshold number.

Real time RT-PCR array for TLR expression

RNA was extracted from human CMF from 3 different donors using the Aurum Total RNA kit (BioRad). Equivalent RNA samples from each of the 3 donors were pooled before preparing cDNA and measuring expression levels of TLRs as part of a SYBR Green-based real-time PCR array (RT^2 Profiler Human Innate and Adaptive Immune Response Array, SABiosciences, Qiagen). Δ C_T values for the target genes were calculated by subtracting the mean C_T value (threshold cycle number) of the 5 housekeeping (HK) genes on the array (β2-microglobulin, hypoxanthine phosphoribosyltransferase 1, ribosomal protein L13A, β-actin and GAPDH) from the C_T value of the target genes. Data are expressed relative to the mean of HK genes using the formula $2^{-\Delta CT}$.

Western blot analysis for TLR4

Whole cell homogenates were prepared from human CMF and cultured human saphenous vein smooth muscle cells, as described previously[28]. Proteins (10 μg) were resolved by SDS-PAGE and immunoblotting performed as described previously[28] with TLR4-specific primary antibody raised in rabbit (#sc10741, Santa Cruz Biotechnology, CA, United States) and horseradish peroxidase-conjugated donkey anti-rabbit secondary antibody (#NA934V, GE Health Care, United Kingdom). Immunolabelled bands were visualised on X-ray film by

SuperSignal West Pico chemiluminescence kit (Perbio, Cramlington, United Kingdom).

Enzyme-linked immunosorbant assay

Cells cultured in 48-well plates were serum-starved for 48 h before exposure to appropriate stimuli for 24 h in a volume of 0.25 mL. Conditioned media were collected and centrifuged to remove cellular debris, and samples were stored at -40 ℃ for subsequent analysis. Enzyme-linked immunosorbant assay for IL-6 was performed according to the manufacturer's instructions (R and D Systems, Abingdon, United Kingdom) using samples diluted 1:100.

Immunocytochemistry for TLR4

Human CMF were fixed in paraformaldehyde (40 g/L) before permeabilising with Triton X-100 (1 mL/L) in PBS. Cells were blocked with bovine serum albumin and incubated with goat primary antibody to human TLR4 (sc-8694, Santa Cruz Biotechnology, CA, United States) followed by Cy3-conjugated donkey anti-goat secondary antibody (1:1000, Stratech Scientific, Soham, Cambridgeshire, United Kingdom). Labelled cells were visualised using a Zeiss Imager. Z1 Apotome microscope and Axiovision image analysis software (Zeiss, Hamburg, Germany). Cells were mounted in Vectashield with DAPI (H-1200, Vector Laboratories, CA, United States). Antibody specificity was confirmed by pre-absorption of primary antibody with TLR4 protein (8 μg/mL for 24 h, sc-8694P, Santa Cruz Biotechnology, CA, United States).

MI model

All procedures involving animals were carried out in accordance with the Home Office Animals (Scientific Procedures) Act 1986 and the University of Leeds Animal Welfare and Ethical Committee. Experiments were performed on C57BL/6 mice (25-30 g, University of Leeds). Animals were maintained at 22 ℃ on a 12 h light and dark cycle with *ad libitum* access to food and water. The animal protocol was designed to minimise pain or discomfort to the animal. Mice were anaesthetised with isofluorane. Under a dissecting microscope, a left thoracotomy was performed at the level of the 4th intercostal space to allow the left anterior descending coronary artery to be visualised. This was ligated at the edge of the left atrium with 8-0 prolene suture. Occlusion was confirmed by observation of pallor of the anterior wall of the LV.

TNC, TRL4 and α-smooth muscle actin immunohistochemistry

Three days following MI, animals were perfused with formaldehyde, hearts were removed and wax-embedded. Tissue sections (3 μm) were cut and stained for both TNC (10337, Immuno-Biological Laboratories Co. Ltd, Japan) and TLR4 (sc-10741, Santa Cruz Biotechnology, CA, United States) using the MenaPath X-Cell Plus Multiplex Double Stain Detection Kit 2 (A.MENARINI Diagnostics, Berkshire, United Kingdom) and a Mouse on Mouse Polymer IHC Kit (Abcam, Cambridge, United Kingdom). Sections were also stained for α smooth muscle actin (clone 1A4, code no. M851; Dakopatts, Glostrup, Denmark) and TLR4 in similar fashion. Tissue sections were counterstained with haematoxylin and imaged using an Axioplan Zeiss microscope and AxioVision 4.8 software (Carl Zeiss Inc.).

Statistical analysis

Results are mean ± SEM with n representing the number of experiments on cells from different patients. Data were analysed using the student's *t*-test or repeated measures one-way ANOVA and Bonferroni multiple comparison post hoc test (GraphPad Prism software, www.graphpad.com). $P < 0.05$ was considered statistically significant. The statistical methods of this study were reviewed by Dr. Emma Spary from the University of Leeds.

RESULTS

Increased expression of TNC and TLR4 in the infarcted ventricle

Three days following LAD ligation, in an experimental mouse model of MI, positive interstitial TNC immunoreactivity (brown) was observed in the infarcted side of the left ventricle but not in the remote non-infarcted regions (Figure 1A and D). Light diffuse TLR4 staining (pink) was noted throughout the myocardium on myocytes and interstitial cells (Figure 1D). On the infarcted side the TLR4 staining was more evident with scattered foci of intense staining visible on myocytes, a feature consistent with previous reports[11] as well as on CMF (Figure 1B and C).

TNC up-regulates IL-6 mRNA and protein expression in human CMF

To determine whether TNC could stimulate MMP or pro-inflammatory cytokine synthesis *in vitro*, human CMF were incubated with TNC (0.1 μmol/L TNC or vehicle for 24 h) and mRNA levels were assessed using RT-PCR. A significant increase (14 fold, $P < 0.01$) in IL-6 mRNA expression from basal levels was observed in response to TNC (Figure 2). TNC also induced an approximately 25-fold increase in MMP3 levels ($P < 0.01$), whereas neither IL-1β nor any of the other MMPs analysed by real-time RT-PCR showed significant changes in mRNA expression in response to TNC (Figure 2). Basal TNFα mRNA expression in human CMF was minimal (0.002% GAPDH) and TNC had no effect on its expression (data not shown).

TLR4 is expressed in human CMF

We examined the mRNA expression of TLR4 in human CMF by RT-PCR and TLR4 protein by both western blot analysis and immunocytochemistry. Real-time RT-PCR

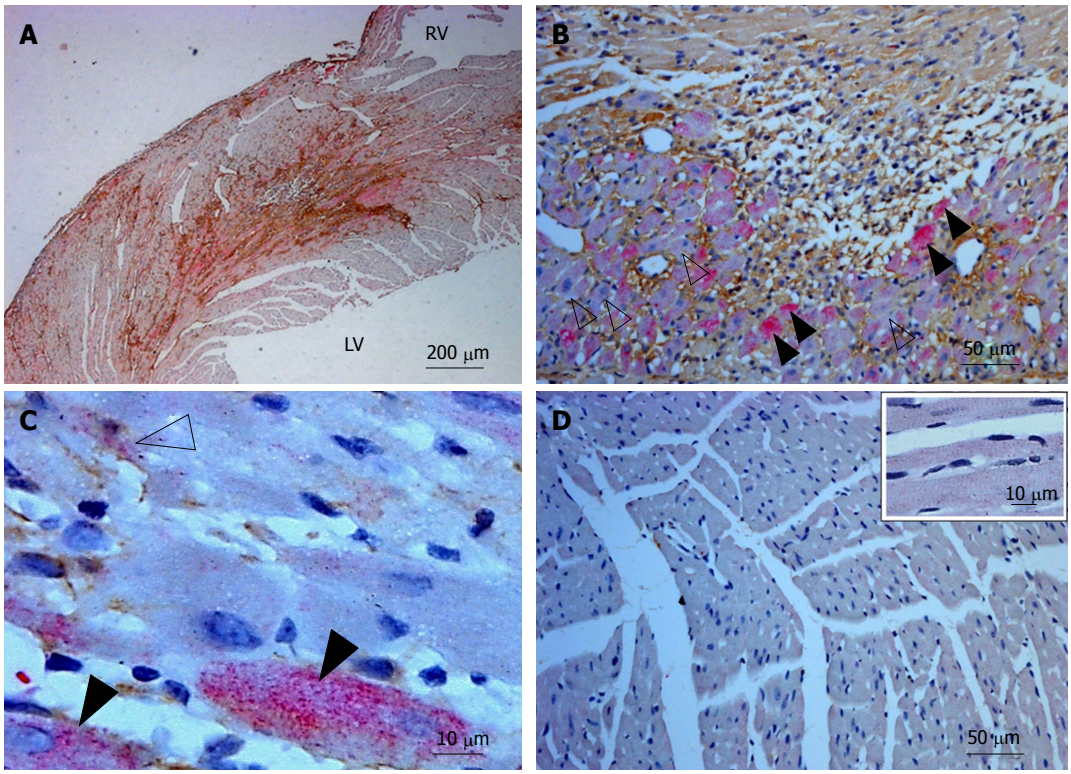

Figure 1 Tenascin C and toll-like receptor 4 staining in the murine left ventricle following infarction. A: Low power view of the infarcted mouse LV showing the proximity of TNC (brown) and TLR4 (pink) 3 d following the occlusion of the left anterior coronary artery; B: Diffuse TLR4 staining (open arrows) in myocytes and interstitial cells and intense TLR4 staining (solid arrows) in some myocytes. Interstitial TNC staining (brown) is evident around these cells; C: High powered view of TLR4 (pink) and alpha smooth muscle actin (brown) staining of cells in the infarcted LV. Intense TLR4 staining can be seen in some myocytes (solid arrow) with more diffuse staining seen in some cardiac myofibroblasts (labelled both pink and brown, open arrow); D: Low power view of the non-infarcted side of the mouse myocardium stained for TNC (brown) and TLR4 (pink). An absence of TNC staining and light diffuse TLR4 staining of the cells is observed. Inset image: a high powered view of cells in this area. In each image cell nuclei were stained with Mayer's Haematoxylin. RV: Right ventricle. LV: Left ventricle; TNC: Tenascin C; TLR4: Toll-like receptor 4.

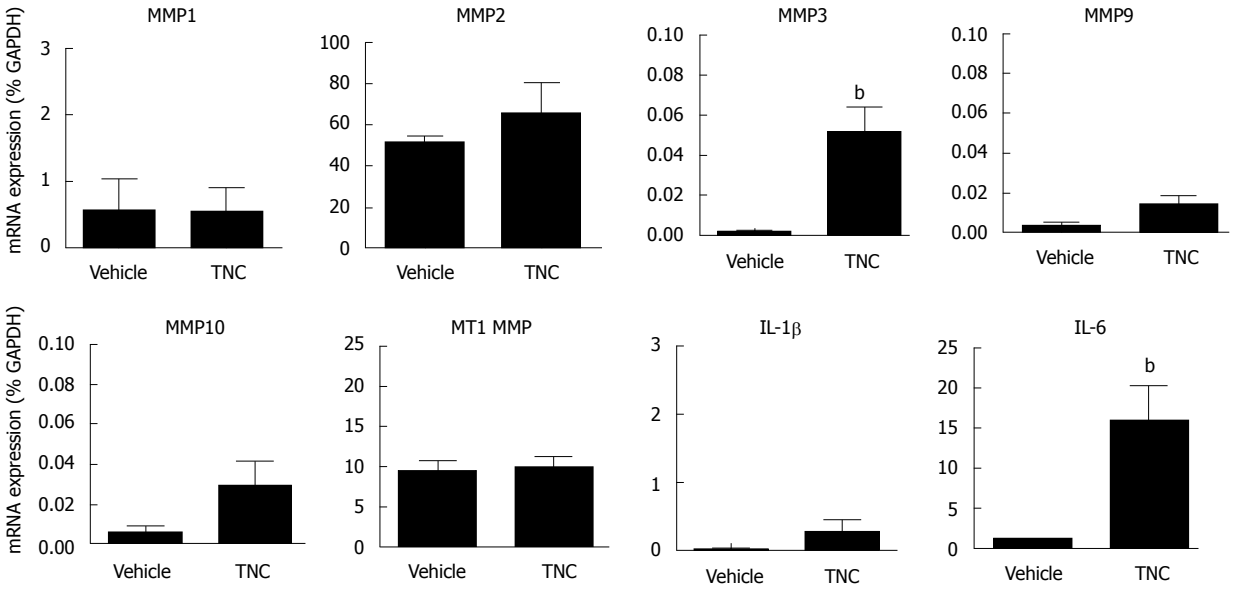

Figure 2 Changes in cytokine and matrix metalloproteinase mRNA expression in human cardiac myofibroblasts following incubation with Tenascin C. Effect of Tenascin C (TNC) (0.1 μmol/L, 24 h) on MMP1, MMP2, MMP3, MMP9, MMP10, MT1-MMP, IL-1β and IL-6 mRNA expression was assessed in human cardiac myofibroblasts (n = 4-6 donors). A significant increase in the expression of MMP3 and IL-6 was observed. Data are expressed as mean ± SEM. $^{b}P < 0.01$ *vs* vehicle (student's *t*-test). MMP: Matrix metalloproteinase; IL: Interleukin.

array analysis of TLRs 1-9 revealed that TLR4 was by far the most highly expressed TLR in human CMF at

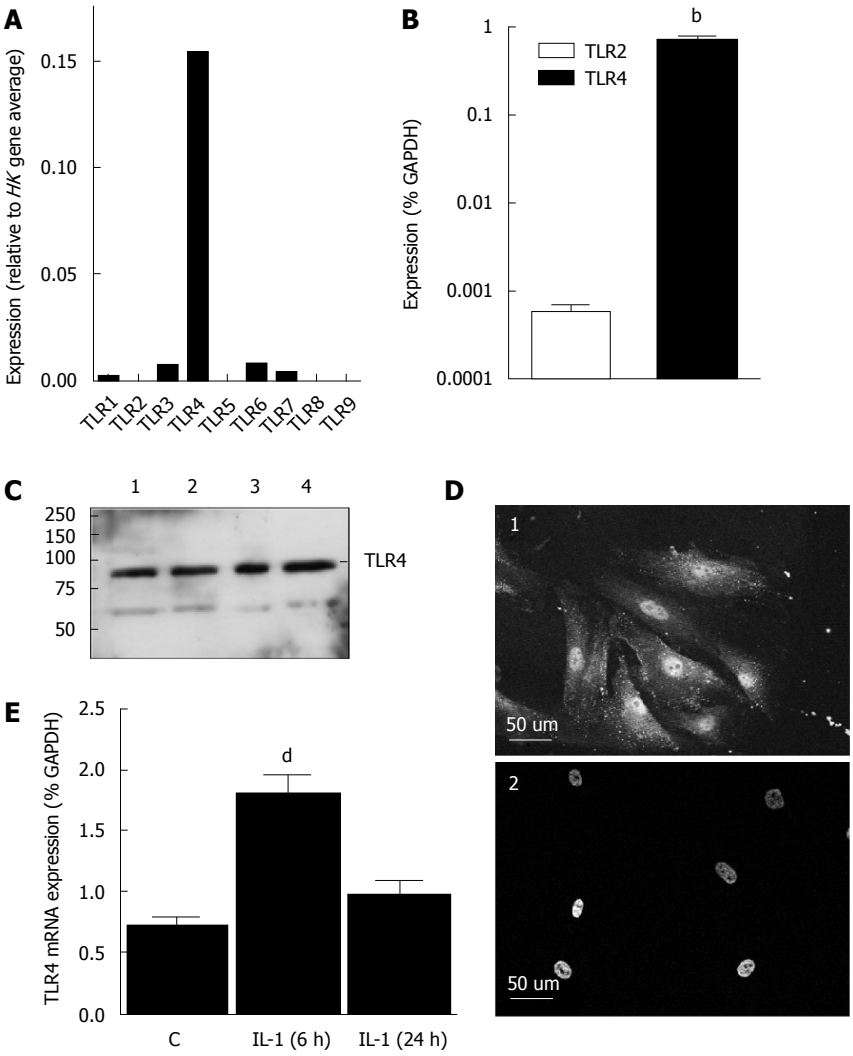

Figure 3 Toll-like receptor 4 expression in human cardiac myofibroblasts. A: Data from RT-PCR array showing abundance of TLR mRNA in a pooled sample of human CMF (n = 3 donors). Data expressed relative to 5 housekeeping genes; B: Taqman RT-PCR analysis of TLR2 and TLR4 mRNA levels in human CMF (n = 4 donors). Note log scale. $^{b}P < 0.001$ (paired t-test); C: Western blot of CMF homogenates from 4 donors probed with anti-TLR4 antibody. Fifteen micrograms protein per lane. Molecular size (kDa) on left. TLR4 = 95 kDa; D1: Immunocytochemical localisation of TLR4 in human CMF using a primary antibody to human TLR4 and a Cy3-conjugated secondary antibody; D2: Effect of pre-absorption of primary antibody with TLR4 peptide (8 μg/mL) prior to immunostaining. Nuclei are labelled with DAPI. Loss of immunostaining confirms specificity of the antibody. Scale bar 50 μm; E: Effect of IL-1α (10 ng/mL, 6 and 24 h) on TLR4 mRNA expression in human CMF (n = 4 donors). Data are expressed as mean ± SEM. $^{d}P < 0.01$ *vs* vehicle (ANOVA post-hoc). TLR: Toll-like receptor.

the mRNA level (Figure 3A). Lower levels of TLR6 > TLR3 > TLR7 > TLR1 were also observed, but TLR2 was not detected. Follow-up studies with Taqman RT-PCR (different primers) on cells from a further 4 donors confirmed the TLR4 and TLR2 data (Figure 3B). Western blot analysis revealed a single 95 kDa band corresponding to TLR4 in protein lysates isolated from human CMF from 4 different donors (Figure 3C). Immunocytochemistry with primary antibodies directed against human TLR4 revealed a pattern of punctate staining in the cytoplasm and nucleus of human CMF (Figure 3D1). Staining was not evident if the primary antibody was pre-incubated with TLR4 protein (Figure 3D2). To assess the effect of inflammatory cytokines on TLR4 mRNA expression, human CMF were incubated with IL-1α. Real-time RT-PCR analysis of TLR4 revealed a 2.5 fold increase of expression after 6 h of treatment, falling back towards basal levels after 24 h (Figure 3E).

TLR4 mediates TNC up-regulation of IL-6 in human CMF

To determine whether the effects of TNC on IL-6 mRNA expression in human CMF were mediated by TLR4, cells from 5 donors were pre-incubated with TLR4 neutralising antibodies prior to TNC treatment (100 nmol/L, 24 h). TLR4 neutralisation had no significant effect on basal IL-6 mRNA expression but did prevent TNC-stimulated expression of IL-6 (Figure 4A). These changes in IL-6 mRNA expression were mirrored by changes in IL-6 protein secretion (Figure 4B). The specificity of the TLR4 antisera for the TLR4 receptor in our *in vitro* studies was confirmed by demonstrating that pre-treating cells with TLR4 neutralising antibodies had no effect on the IL-1α induced IL-6 mRNA expression (Figure 4C).

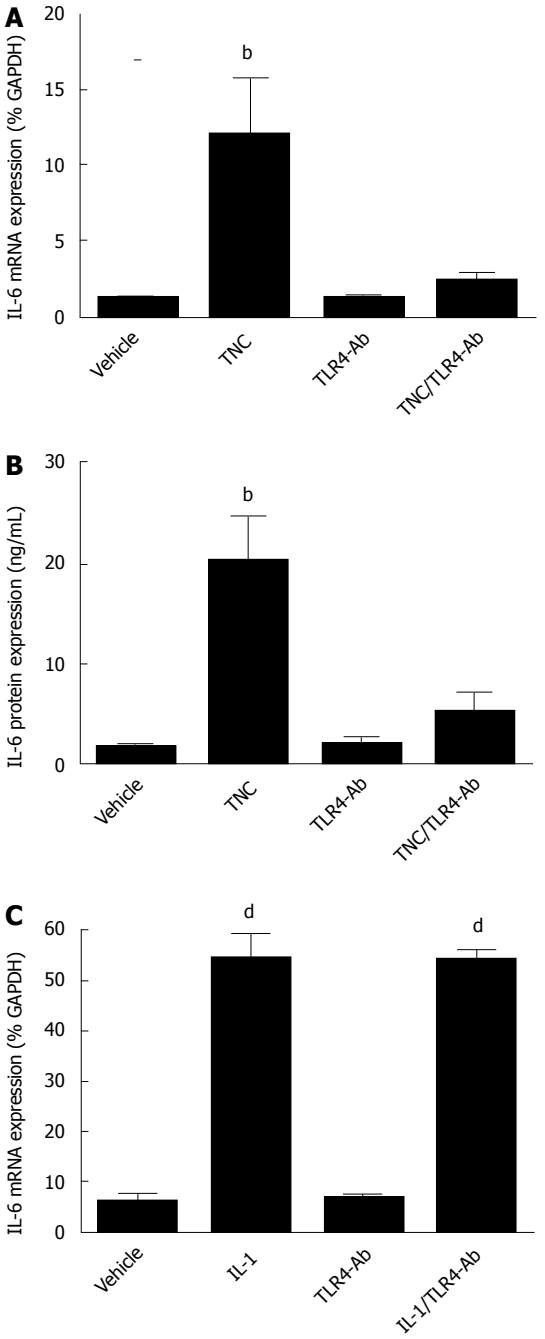

Figure 4 Tenascin C upregulates interleukin-6 expression in human cardiac myofibroblasts *via* toll-like receptor 4. A and B: Effect of TNC (0.1 μmol/L, 24 h) with and without 1 h pre-incubation in TLR4 antisera (25 μg/mL) on IL-6 mRNA (A) and IL-6 protein expression (B) in CMF (4-5 donors). Data expressed as mean ± SEM. $^{b}P < 0.01$ *vs* vehicle (ANOVA post-hoc). TNC: Stimulation with TNC alone; TLR4-Ab: Pre-incubation with TLR4 neutralising antisera; TNC/TLR4-Ab: Stimulation with TNC following TLR4 pre-incubation; C: Effect of 1 h pre-incubation in TLR4 antisera (25 μg/mL) on IL-1α (10 ng/mL, 24 h)-stimulated IL-6 mRNA expression in CMF (n = 3 donors). The TLR4 neutralising antisera had no effect on IL-1α induced IL-6mRNA expression. Data expressed as mean ± SEM. $^{d}P < 0.0001$ *vs* vehicle (ANOVA post-hoc). IL-1: Stimulation with IL-1α alone; TLR4-Ab: Pre-incubation with TLR4 neutralising antisera; IL-1/TLR4-Ab: Stimulation with IL-1α following TLR4 pre-incubation; TNC: Tenascin C; CMF: Cardiac myofibroblasts; IL: Interleukin; TLR: Toll-like receptor.

FBG domain upregulates IL-6 mRNA expression in human CMF

Previous studies have implicated the FBG domain of TNC in the promotion of cytokine production in synovial fibroblasts[14]. To determine whether a similar effect was evident in human CMF, cells were incubated with recombinant FBG protein (1 μmol/L, 24 h) and IL-6 mRNA expression assessed. FBG induced a 6-fold increase in IL-6 mRNA expression ($P < 0.01$), whereas incubation with the TNⅢ 5-7 recombinant TNC fragment had no effect, endorsing the suggestion that the FBG domain was crucial for promoting IL-6 production (Figure 5A). Neither FBG nor TNⅢ 5-7 recombinant proteins significantly increased MMP-3 expression in human CMF (Figure 5B).

TLR4 mediates FBG up-regulation of IL-6 in human CMF

To determine whether the effects of FBG on IL-6 mRNA expression in human CMF were mediated by TLR4 pathways, cells from 3 donors were pre-incubated with TLR4 neutralising antibodies prior to FBG treatment (1 μmol/L, 24 h). TLR4 antibodies had no significant effect on basal IL-6 mRNA expression but did prevent FBG -stimulated expression of IL-6 (Figure 5C).

DISCUSSION

The main finding of the present study is that TNC up-regulates IL-6 expression in human CMF and that this effect is mediated through its FBG domain and the TLR4 receptor.

Inflammation and matrix turnover are important features in cardiac remodelling post infarction. If unchecked, these can lead to chronic maladaptive LV remodelling, characterised by progressive ventricular dilatation, myocardial hypertrophy, fibrosis, cardiac dysfunction and failure. TNC is an ECM glycoprotein that is re-expressed in the heart under pathological conditions such as MI[29,30], myocarditis[3,31,32] and dilated cardiomyopathy[4,33] and is closely associated with tissue injury and inflammation[3,31,34]. Following MI, TNC has been reported to appear during the acute stage at the interface between the infarcted and intact myocardium where tissue remodelling is at its most active[2]. There is evidence that TNC is a sensitive marker for active inflammation in acute myocarditis[3]. Moreover, serum levels of TNC correlate with the severity of heart failure, LV dysfunction and remodelling in patients with dilated cardiomyopathy[35]. These observations suggest that TNC expression is maintained in cardiac pathologies in which there is ongoing inflammation and remodelling.

We previously reported that IL-1α up-regulates TNC expression in human CMF[11]. IL-1 has been identified as one of the initial stimuli that drive the acute inflammatory response following MI[36-39]. Increased levels of IL-1 have also been implicated in the inflammatory response and adverse LV remodelling seen in heart failure[40]. The observation that TNC up-regulates IL-6 expression in our present study supports the previous notion that after its initial induction TNC could drive the inflammatory response further[14].

IL-6 has previously been shown to have important roles in inflammation and remodelling in the heart

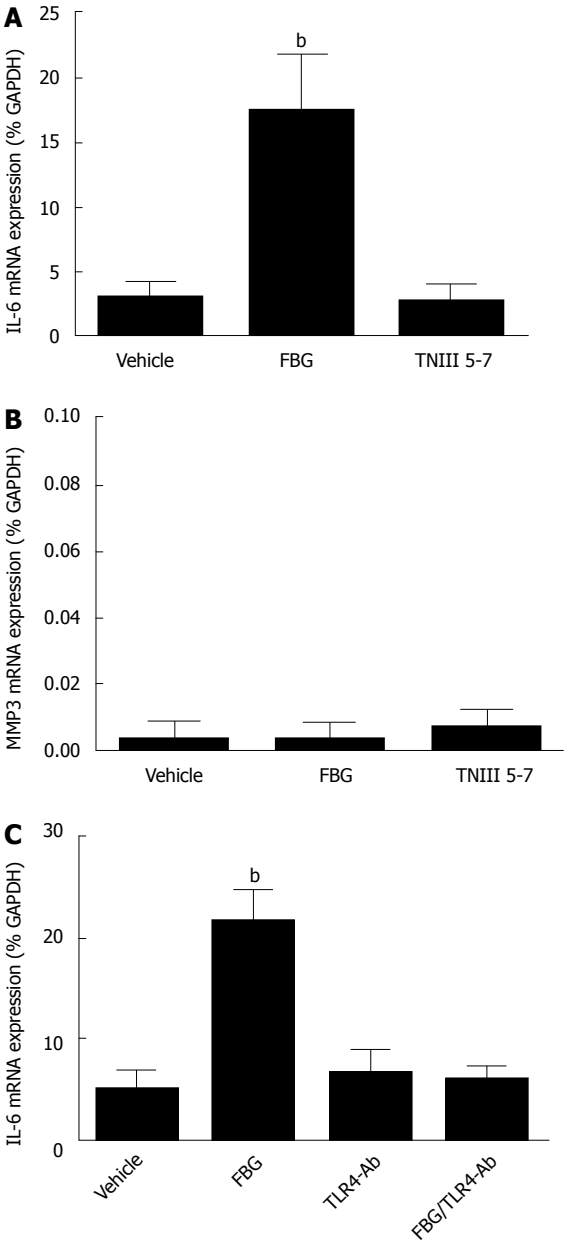

Figure 5 Fibrinogen-like globe domain of Tenascin C up-regulates interleukin-6 mRNA expression in human cardiac myofibroblasts *via* toll-like receptor 4. A and B: Effect of 1 μmol/L (24 h) recombinant protein, corresponding to either the FBG domain or the fibronectin type III domain (TNIII 5-7) of TNC, on IL-6 mRNA (A) and MMP3 mRNA (B) in human CMF (n = 3-5 donors). Data expressed as mean ± SEM. $^{b}P < 0.01$ *vs* vehicle (ANOVA post-hoc); C: Effect of 1 h pre-incubation in TLR4 neutralising antisera (25 μg/mL) alone on IL-6 mRNA expression and on FBG (1 μmol/L, 24 h) stimulated IL-6 mRNA expression in CMF (n = 3 donors). Data expressed as mean ± SEM. $^{b}P < 0.01$ *vs* vehicle (ANOVA post-hoc). FBG: Stimulation with FBG recombinant alone; TLR4-Ab: Pre-incubation with TLR4 neutralising antisera; FBG/TLR4-Ab: Stimulation with FBG following pre-incubation with TLR4 neutralising antisera. FBG: Fibrinogen-like globe; TNC: Tenascin C; IL: Interleukin; CMF: Cardiac myofibroblasts.

following injury by promoting leukocyte infiltration and activation, and by modulating fibroblast function[23,41-43]. In addition to its pro-inflammatory and fibrogenic properties, there is also evidence that IL-6 regulates ECM remodelling by enhancing cardiac fibroblast MMP expression and modulating tissue inhibitor of MMPs (TIMP) expression levels[23,42,44]. Our observations are consistent with a previous report in synovial fibroblasts, where an up-regulation of IL-6 expression following TNC stimulation was purported to play a role in the chronic inflammatory response seen in the arthritic joint[14]. In that study, the TLR4 pathway was identified as a mediator of this effect with the TLR4 receptor being activated by the FBG domain of TNC. Importantly, our study suggests that this notable mechanism may also play a role in the inflammatory response in the heart. As TLR4 signalling has long been implicated in a range of cardiac pathologies, the confirmation of such a mechanism in cardiac cells contributes to our understanding of the process of inflammation that occurs in the heart.

A regulatory role for TLR4 signalling in inflammation and ECM turnover has been established and its involvement in post-infarct maladaptive remodelling in the heart has been reported[1]. Moreover, TLR4 signalling has been implicated in myocarditis[45] and in the myocardial inflammatory response following regional or global ischemia/reperfusion[20,22,46,47]. Although TLR4 signalling contributes to cardiac dysfunction after MI in part through its influence on myocardial production of cytokines, the mechanism by which TLR4 is activated in these circumstances remains to be defined[22]. Evidence that TNC is up-regulated following oxidative stress suggests it may be a candidate ligand responsible for TLR4 signalling following ischemic injury[48]. Furthermore, our observations that the inflammatory cytokine, IL-1α, upregulates both TNC[11] and TLR4 expression (Figure 3E) in CMF and that both TNC and TLR4 are upregulated in the infarcted ventricle (Figure 1) lends support to their involvement in the cardiac inflammatory response following MI[2,14,49].

With the exception of MMP3, neither IL-1β nor any of the MMPs analysed by real-time RT-PCR showed significant changes in mRNA expression in response to TNC. In the case of MMP3, although a significant increase from basal expression was indeed observed with TNC, the mean induced MMP3 levels were only 0.05% that of the *HK* gene GAPDH. Nevertheless, this is equivalent to the mRNA level observed in response to a low concentration of IL-1 (0.1 ng/mL) in these cells which resulted in detectable MMP3 protein secretion[50]. We found no evidence, however, that FBG was responsible for the increase in MMP3 observed. The possibility that a different domain of this molecule, other than FBG or TNⅢ5-7, plays a role in MMP3 induction remains to be tested. Moreover, the recent demonstration that some of the effects of TNC are mediated *via* αVβ3 integrin, suggests that the induction of MMP3 by TNC may occur *via* a different receptor subtype[51].

The up-regulation of MMP3 by TNC would be consistent with TNC's role in the degradation of ECM following MI and the promotion of a de-adhesive state that facilitates migration of fibroblasts and other granulation tissue cells to the site of injury[52]. TNC has previously

been reported to induce cell-specific increases in MMP levels including cultured human smooth muscle cells, murine mammary cancer cells, macrophages and synovial fibroblasts[53-56].

Finally, the lack of induction of IL-1β and TNFα by TNC is consistent with previous studies which have reported that stimulation of synovial fibroblasts with TNC resulted in augmented gene expression of some pro-inflammatory cytokines (*e.g.*, IL-1α and IL-6), but not others (*e.g.*, interferon-γ, TNF-α and IL-1β)[14,56].

In conclusion, we have demonstrated that TNC up-regulates MMP3 and the pro-inflammatory cytokine IL-6 in human CMF. The latter effect of TNC is mediated *via* the TLR4 receptor and the FBG domain of the TNC. Targeting the FBG domain of TNC may provide a novel therapeutic option to counteract aberrant inflammation and maladaptive cardiac remodelling.

ACKNOWLEDGMENTS

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COMMENTS

Background

Adverse ventricular remodelling following cardiac injury is a key determinant of heart failure. Despite major advances in the field, the number of heart failure deaths is increasing steadily. A better understanding of the processes involved in the remodelling process would enable the development of novel therapeutics.

Research frontiers

The importance of inflammation in cardiac remodelling that occurs after myocardial injury is unequivocal. The inflammatory process can cause myocardial damage, while inflammatory agents contribute to the worsening and progression of heart failure. A detailed understanding of the underlying inflammatory processes involved in cardiac remodelling, with the aim to develop better therapeutics, remains an important area in heart failure research.

Innovation and breakthroughs

Tenascin C (TNC) is highly expressed during embryogenesis but is virtually absent in the adult heart. It is re-expressed in the heart following injury where its expression correlates with the extent of inflammation and myocardial remodelling. The continued expression of TNC in pathologies associated with cardiac inflammation (*e.g.*, myocarditis, heart failure) had been recognised yet its functional significance remained elusive. This study is the first to demonstrate that TNC is able to stimulate the expression of the inflammatory cytokine interleukin (IL)-6 in human cardiac myofibroblasts (CMF) in a toll-like receptor 4-dependent fashion. This action is similar to that reported in the arthritic joint where TNC contributes to the persistent inflammation observed. As IL-6 plays an important role in myocardial inflammation and fibrosis, identification of this notable mechanism in CMF will further our understanding of the inflammatory processes occurring after myocardial stress or injury.

Application

The results provide further insight into the underlying mechanism(s) involved in cardiac inflammation and may help identify novel therapeutic targets to attenuate this in disease states.

Peer-review

This manuscript reports the effects of TNC on the expression of the pro-inflammatory cytokines in human CMF. The findings are of interest and suitable for the publication by the journal.

REFERENCES

1 **Timmers L**, Sluijter JP, van Keulen JK, Hoefer IE, Nederhoff MG, Goumans MJ, Doevendans PA, van Echteld CJ, Joles JA, Quax PH, Piek JJ, Pasterkamp G, de Kleijn DP. Toll-like receptor 4 mediates maladaptive left ventricular remodeling and impairs cardiac function after myocardial infarction. *Circ Res* 2008; **102**: 257-264 [PMID: 18007026 DOI: 10.1161/circresaha.107.158220]

2 **Imanaka-Yoshida K**, Hiroe M, Nishikawa T, Ishiyama S, Shimojo T, Ohta Y, Sakakura T, Yoshida T. Tenascin-C modulates adhesion of cardiomyocytes to extracellular matrix during tissue remodeling after myocardial infarction. *Lab Invest* 2001; **81**: 1015-1024 [PMID: 11454990 DOI: 10.1038/labinvest.3780313]

3 **Morimoto S**, Imanaka-Yoshida K, Hiramitsu S, Kato S, Ohtsuki M, Uemura A, Kato Y, Nishikawa T, Toyozaki T, Hishida H, Yoshida T, Hiroe M. Diagnostic utility of tenascin-C for evaluation of the activity of human acute myocarditis. *J Pathol* 2005; **205**: 460-467 [PMID: 15685595 DOI: 10.1002/path.1730]

4 **Tamura A**, Kusachi S, Nogami K, Yamanishi A, Kajikawa Y, Hirohata S, Tsuji T. Tenascin expression in endomyocardial biopsy specimens in patients with dilated cardiomyopathy: distribution along margin of fibrotic lesions. *Heart* 1996; **75**: 291-294 [PMID: 8800995 DOI: 10.1136/hrt.75.3.291]

5 **Rettig WJ**, Erickson HP, Albino AP, Garin-Chesa P. Induction of human tenascin (neuronectin) by growth factors and cytokines: cell type-specific signals and signalling pathways. *J Cell Sci* 1994; **107** (Pt 2): 487-497 [PMID: 7515896]

6 **Chiquet M**. Regulation of extracellular matrix gene expression by mechanical stress. *Matrix Biol* 1999; **18**: 417-426 [PMID: 10601729 DOI: 10.1016/s0945-053x(99)00039-6]

7 **Yamamoto K**, Dang QN, Kennedy SP, Osathanondh R, Kelly RA, Lee RT. Induction of tenascin-C in cardiac myocytes by mechanical deformation. Role of reactive oxygen species. *J Biol Chem* 1999; **274**: 21840-21846 [PMID: 10419501 DOI: 10.1074/jbc.274.31.21840]

8 **Järvinen TA**, Józsa L, Kannus P, Järvinen TL, Hurme T, Kvist M, Pelto-Huikko M, Kalimo H, Järvinen M. Mechanical loading regulates the expression of tenascin-C in the myotendinous junction and tendon but does not induce de novo synthesis in the skeletal muscle. *J Cell Sci* 2003; **116**: 857-866 [PMID: 12571283 DOI: 10.1242/jcs.00303]

9 **Chiquet M**, Sarasa-Renedo A, Tunç-Civelek V. Induction of tenascin-C by cyclic tensile strain versus growth factors: distinct contributions by Rho/ROCK and MAPK signaling pathways. *Biochim Biophys Acta* 2004; **1693**: 193-204 [PMID: 15363633 DOI: 10.1016/j.bbamcr.2004.08.001]

10 **Ogawa K**, Ito M, Takeuchi K, Nakada A, Heishi M, Suto H, Mitsuishi K, Sugita Y, Ogawa H, Ra C. Tenascin-C is upregulated in the skin lesions of patients with atopic dermatitis. *J Dermatol Sci* 2005; **40**: 35-41 [PMID: 16043328 DOI: 10.1016/j.jdermsci.2005.06.001]

11 **Maqbool A**, Hemmings KE, O'Regan DJ, Ball SG, Porter KE, Turner NA. Interleukin-1 has opposing effects on connective tissue growth factor and tenascin-C expression in human cardiac fibroblasts. *Matrix Biol* 2013; **32**: 208-214 [PMID: 23454256 DOI: 10.1016/j.matbio.2013.02.003]

12 **Tamaoki M**, Imanaka-Yoshida K, Yokoyama K, Nishioka T, Inada H, Hiroe M, Sakakura T, Yoshida T. Tenascin-C regulates recruitment of myofibroblasts during tissue repair after myocardial injury. *Am J Pathol* 2005; **167**: 71-80 [PMID: 15972953 DOI: 10.1016/s0002-9440(10)62954-9]

13 **Imanaka-Yoshida K**, Hiroe M, Yoshida T. Interaction between cell and extracellular matrix in heart disease: multiple roles of tenascin-C in tissue remodeling. *Histol Histopathol* 2004; **19**: 517-525 [PMID: 15024713]

14 **Midwood K**, Sacre S, Piccinini AM, Inglis J, Trebaul A, Chan E, Drexler S, Sofat N, Kashiwagi M, Orend G, Brennan F, Foxwell B. Tenascin-C is an endogenous activator of Toll-like receptor 4 that is essential for maintaining inflammation in arthritic joint disease. *Nat*

Med 2009; **15**: 774-780 [PMID: 19561617 DOI: 10.1038/nm.1987]
15 **Medzhitov R**, Preston-Hurlburt P, Janeway CA. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* 1997; **388**: 394-397 [PMID: 9237759 DOI: 10.1038/41131]
16 **Akira S**, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2001; **2**: 675-680 [PMID: 11477402 DOI: 10.1038/90609]
17 **Baumgarten G**, Knuefermann P, Nozaki N, Sivasubramanian N, Mann DL, Vallejo JG. In vivo expression of proinflammatory mediators in the adult heart after endotoxin administration: the role of toll-like receptor-4. *J Infect Dis* 2001; **183**: 1617-1624 [PMID: 11343210 DOI: 10.1086/320712]
18 **Monaco C**, Gregan SM, Navin TJ, Foxwell BM, Davies AH, Feldmann M. Toll-like receptor-2 mediates inflammation and matrix degradation in human atherosclerosis. *Circulation* 2009; **120**: 2462-2469 [PMID: 19948979 DOI: 10.1161/circulationaha.109.851881]
19 **Frantz S**, Kobzik L, Kim YD, Fukazawa R, Medzhitov R, Lee RT, Kelly RA. Toll4 (TLR4) expression in cardiac myocytes in normal and failing myocardium. *J Clin Invest* 1999; **104**: 271-280 [PMID: 10430608 DOI: 10.1172/jci6709]
20 **Chong AJ**, Shimamoto A, Hampton CR, Takayama H, Spring DJ, Rothnie CL, Yada M, Pohlman TH, Verrier ED. Toll-like receptor 4 mediates ischemia/reperfusion injury of the heart. *J Thorac Cardiovasc Surg* 2004; **128**: 170-179 [PMID: 15282452 DOI: 10.1016/j.jtcvs.2003.11.036]
21 **Shimamoto A**, Chong AJ, Yada M, Shomura S, Takayama H, Fleisig AJ, Agnew ML, Hampton CR, Rothnie CL, Spring DJ, Pohlman TH, Shimpo H, Verrier ED. Inhibition of Toll-like receptor 4 with eritoran attenuates myocardial ischemia-reperfusion injury. *Circulation* 2006; **114**: I270-I274 [PMID: 16820585 DOI: 10.1161/circulationaha.105.000901]
22 **Cha J**, Wang Z, Ao L, Zou N, Dinarello CA, Banerjee A, Fullerton DA, Meng X. Cytokines link Toll-like receptor 4 signaling to cardiac dysfunction after global myocardial ischemia. *Ann Thorac Surg* 2008; **85**: 1678-1685 [PMID: 18442564 DOI: 10.1016/j.athoracsur.2008.01.043]
23 **Porter KE**, Turner NA. Cardiac fibroblasts: at the heart of myocardial remodeling. *Pharmacol Ther* 2009; **123**: 255-278 [PMID: 19460403 DOI: 10.1016/j.pharmthera.2009.05.002]
24 **Turner NA**, Porter KE. Function and fate of myofibroblasts after myocardial infarction. *Fibrogenesis Tissue Repair* 2013; **6**: 5 [PMID: 23448358 DOI: 10.1186/1755-1536-6-5]
25 **Porter KE**, Turner NA, O'Regan DJ, Ball SG. Tumor necrosis factor alpha induces human atrial myofibroblast proliferation, invasion and MMP-9 secretion: inhibition by simvastatin. *Cardiovasc Res* 2004; **64**: 507-515 [PMID: 15537504 DOI: 10.1016/j.cardiores.2004.07.020]
26 **Mughal RS**, Warburton P, O'Regan DJ, Ball SG, Turner NA, Porter KE. Peroxisome proliferator-activated receptor gamma-independent effects of thiazolidinediones on human cardiac myofibroblast function. *Clin Exp Pharmacol Physiol* 2009; **36**: 478-486 [PMID: 19673929 DOI: 10.1111/j.1440-1681.2008.05088.x]
27 **Turner NA**, Mughal RS, Warburton P, O'Regan DJ, Ball SG, Porter KE. Mechanism of TNFalpha-induced IL-1alpha, IL-1beta and IL-6 expression in human cardiac fibroblasts: effects of statins and thiazolidinediones. *Cardiovasc Res* 2007; **76**: 81-90 [PMID: 17612514 DOI: 10.1016/j.cardiores.2007.06.003]
28 **Turner NA**, Ball SG, Balmforth AJ. The mechanism of angiotensin II-induced extracellular signal-regulated kinase-1/2 activation is independent of angiotensin AT(1A) receptor internalisation. *Cell Signal* 2001; **13**: 269-277 [PMID: 11306244 DOI: 10.1016/s0898-6568(01)00135-8]
29 **Smoak KA**, Aloor JJ, Madenspacher J, Merrick BA, Collins JB, Zhu X, Cavigiolio G, Oda MN, Parks JS, Fessler MB. Myeloid differentiation primary response protein 88 couples reverse cholesterol transport to inflammation. *Cell Metab* 2010; **11**: 493-502 [PMID: 20519121 DOI: 10.1016/j.cmet.2010.04.006]
30 **Willems IE**, Arends JW, Daemen MJ. Tenascin and fibronectin expression in healing human myocardial scars. *J Pathol* 1996; **179**: 321-325 [PMID: 8774490 DOI: 10.1002/(sici)1096-9896(199607)179: 3<321: : aid-path555>3.3.co; 2-#]
31 **Imanaka-Yoshida K**, Hiroe M, Yasutomi Y, Toyozaki T, Tsuchiya T, Noda N, Maki T, Nishikawa T, Sakakura T, Yoshida T. Tenascin-C is a useful marker for disease activity in myocarditis. *J Pathol* 2002; **197**: 388-394 [PMID: 12115886 DOI: 10.1002/path.1131]
32 **Sato M**, Toyozaki T, Odaka K, Uehara T, Arano Y, Hasegawa H, Yoshida K, Imanaka-Yoshida K, Yoshida T, Hiroe M, Tadokoro H, Irie T, Tanada S, Komuro I. Detection of experimental autoimmune myocarditis in rats by 111In monoclonal antibody specific for tenascin-C. *Circulation* 2002; **106**: 1397-1402 [PMID: 12221059 DOI: 10.1161/01.cir.0000027823.07104.86]
33 **Tsukada B**, Terasaki F, Shimomura H, Otsuka K, Otsuka K, Katashima T, Fujita S, Imanaka-Yoshida K, Yoshida T, Hiroe M, Kitaura Y. High prevalence of chronic myocarditis in dilated cardiomyopathy referred for left ventriculoplasty: expression of tenascin C as a possible marker for inflammation. *Hum Pathol* 2009; **40**: 1015-1022 [PMID: 19297005 DOI: 10.1016/j.humpath.2008.12.017]
34 **Nishioka T**, Suzuki M, Onishi K, Takakura N, Inada H, Yoshida T, Hiroe M, Imanaka-Yoshida K. Eplerenone attenuates myocardial fibrosis in the angiotensin II-induced hypertensive mouse: involvement of tenascin-C induced by aldosterone-mediated inflammation. *J Cardiovasc Pharmacol* 2007; **49**: 261-268 [PMID: 17513943 DOI: 10.1097/fjc.0b013e318033dfd4]
35 **Terasaki F**, Okamoto H, Onishi K, Sato A, Shimomura H, Tsukada B, Imanaka-Yoshida K, Hiroe M, Yoshida T, Kitaura Y, Kitabatake A. Higher serum tenascin-C levels reflect the severity of heart failure, left ventricular dysfunction and remodeling in patients with dilated cardiomyopathy. *Circ J* 2007; **71**: 327-330 [PMID: 17322629 DOI: 10.1253/circj.71.327]
36 **Dinarello CA**, Pomerantz BJ. Proinflammatory cytokines in heart disease. *Blood Purif* 2001; **19**: 314-321 [PMID: 11244192 DOI: 10.1159/000046960]
37 **Deten A**, Volz HC, Briest W, Zimmer HG. Cardiac cytokine expression is upregulated in the acute phase after myocardial infarction. Experimental studies in rats. *Cardiovasc Res* 2002; **55**: 329-340 [PMID: 12123772 DOI: 10.1016/s0008-6363(02)00413-3]
38 **Turner NA**, Das A, Warburton P, O'Regan DJ, Ball SG, Porter KE. Interleukin-1alpha stimulates proinflammatory cytokine expression in human cardiac myofibroblasts. *Am J Physiol Heart Circ Physiol* 2009; **297**: H1117-H1127 [PMID: 19648252 DOI: 10.1152/ajpheart.00372.2009]
39 **Turner NA**. Effects of interleukin-1 on cardiac fibroblast function: relevance to post-myocardial infarction remodelling. *Vascul Pharmacol* 2014; **60**: 1-7 [PMID: 23806284 DOI: 10.1016/j.vph.2013.06.002]
40 **Mann DL**. Inflammatory mediators and the failing heart: past, present, and the foreseeable future. *Circ Res* 2002; **91**: 988-998 [PMID: 12456484 DOI: 10.1161/01.res.0000043825.01705.1b]
41 **Jones SA**. Directing transition from innate to acquired immunity: defining a role for IL-6. *J Immunol* 2005; **175**: 3463-3468 [PMID: 16148087 DOI: 10.4049/jimmunol.175.6.3463]
42 **Bujak M**, Dobaczewski M, Chatila K, Mendoza LH, Li N, Reddy A, Frangogiannis NG. Interleukin-1 receptor type I signaling critically regulates infarct healing and cardiac remodeling. *Am J Pathol* 2008; **173**: 57-67 [PMID: 18535174 DOI: 10.2353/ajpath.2008.070974]
43 **Nowell MA**, Williams AS, Carty SA, Scheller J, Hayes AJ, Jones GW, Richards PJ, Slinn S, Ernst M, Jenkins BJ, Topley N, Rose-John S, Jones SA. Therapeutic targeting of IL-6 trans signaling counteracts STAT3 control of experimental inflammatory arthritis. *J Immunol* 2009; **182**: 613-622 [PMID: 19109195 DOI: 10.4049/jimmunol.182.1.613]
44 **Kossakowska AE**, Edwards DR, Prusinkiewicz C, Zhang MC, Guo D, Urbanski SJ, Grogan T, Marquez LA, Janowska-Wieczorek A. Interleukin-6 regulation of matrix metalloproteinase (MMP-2 and MMP-9) and tissue inhibitor of metalloproteinase (TIMP-1) expression in malignant non-Hodgkin's lymphomas. *Blood* 1999; **94**: 2080-2089 [PMID: 10477738]

45 **Fairweather D**, Yusung S, Frisancho S, Barrett M, Gatewood S, Steele R, Rose NR. IL-12 receptor beta 1 and Toll-like receptor 4 increase IL-1 beta- and IL-18-associated myocarditis and coxsackievirus replication. *J Immunol* 2003; **170**: 4731-4737 [PMID: 12707353 DOI: 10.4049/jimmunol.170.9.4731]

46 **Oyama J**, Blais C, Liu X, Pu M, Kobzik L, Kelly RA, Bourcier T. Reduced myocardial ischemia-reperfusion injury in toll-like receptor 4-deficient mice. *Circulation* 2004; **109**: 784-789 [PMID: 14970116 DOI: 10.1161/01.cir.0000112575.66565.84]

47 **Stapel H**, Kim SC, Osterkamp S, Knuefermann P, Hoeft A, Meyer R, Grohé C, Baumgarten G. Toll-like receptor 4 modulates myocardial ischaemia-reperfusion injury: Role of matrix metalloproteinases. *Eur J Heart Fail* 2006; **8**: 665-672 [PMID: 16829192 DOI: 10.1016/j.ejheart.2006.03.005]

48 **Taki J**, Inaki A, Wakabayashi H, Imanaka-Yoshida K, Ogawa K, Hiroe M, Shiba K, Yoshida T, Kinuya S. Dynamic expression of tenascin-C after myocardial ischemia and reperfusion: assessment by 125I-anti-tenascin-C antibody imaging. *J Nucl Med* 2010; **51**: 1116-1122 [PMID: 20554738 DOI: 10.2967/jnumed.109.071340]

49 **Turner NA**. Inflammatory and fibrotic responses of cardiac fibroblasts to myocardial damage associated molecular patterns (DAMPs). *J Mol Cell Cardiol* 2016; **94**: 189-200 [PMID: 26542796 DOI: 10.1016/j.yjmcc.2015.11.002]

50 **van Nieuwenhoven FA**, Hemmings KE, Porter KE, Turner NA. Combined effects of interleukin-1α and transforming growth factor-β1 on modulation of human cardiac fibroblast function. *Matrix Biol* 2013; **32**: 399-406 [PMID: 23583823 DOI: 10.1016/j.matbio.2013.03.008]

51 **Shimojo N**, Hashizume R, Kanayama K, Hara M, Suzuki Y, Nishioka T, Hiroe M, Yoshida T, Imanaka-Yoshida K. Tenascin-C may accelerate cardiac fibrosis by activating macrophages via the integrin αVβ3/nuclear factor-κB/interleukin-6 axis. *Hypertension* 2015; **66**: 757-766 [PMID: 26238448 DOI: 10.1161/HYPERTENSIONAHA.115.06004]

52 **Dobaczewski M**, Gonzalez-Quesada C, Frangogiannis NG. The extracellular matrix as a modulator of the inflammatory and reparative response following myocardial infarction. *J Mol Cell Cardiol* 2010; **48**: 504-511 [PMID: 19631653 DOI: 10.1016/j.yjmcc.2009.07.015]

53 **Wallner K**, Li C, Shah PK, Wu KJ, Schwartz SM, Sharifi BG. EGF-Like domain of tenascin-C is proapoptotic for cultured smooth muscle cells. *Arterioscler Thromb Vasc Biol* 2004; **24**: 1416-1421 [PMID: 15178565 DOI: 10.1161/01.atv.0000134299.89599.53]

54 **Kalembeyi I**, Inada H, Nishiura R, Imanaka-Yoshida K, Sakakura T, Yoshida T. Tenascin-C upregulates matrix metalloproteinase-9 in breast cancer cells: direct and synergistic effects with transforming growth factor beta1. *Int J Cancer* 2003; **105**: 53-60 [PMID: 12672030 DOI: 10.1002/ijc.11037]

55 **Khan KM**, Falcone DJ. Role of laminin in matrix induction of macrophage urokinase-type plasminogen activator and 92-kDa metalloproteinase expression. *J Biol Chem* 1997; **272**: 8270-8275 [PMID: 9079647 DOI: 10.1074/jbc.272.13.8270]

56 **Kanayama M**, Kurotaki D, Morimoto J, Asano T, Matsui Y, Nakayama Y, Saito Y, Ito K, Kimura C, Iwasaki N, Suzuki K, Harada T, Li HM, Uehara J, Miyazaki T, Minami A, Kon S, Uede T. Alpha9 integrin and its ligands constitute critical joint microenvironments for development of autoimmune arthritis. *J Immunol* 2009; **182**: 8015-8025 [PMID: 19494327 DOI: 10.4049/jimmunol.0900725]

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CASE REPORT

Relationship between coronary artery ectasia, cocaine abuse and acute coronary syndromes

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Author contributions: Dendramis G designed the report, analyzed the data and wrote the paper; Paleologo C collected the patient's clinical data; Piraino D and Assennato P analyzed the data.

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Abstract

Coronary artery ectasia (CAE) often represents a coronary angiography finding casually detected or following the occurrence of an acute coronary syndrome. The pathogenetic role of cocaine abuse in the genesis of CAE is still little known and very few data are available in literature. We describe a case of a 31-year-old male cocaine user admitted to our department for typical acute chest pain. Coronary angiography showed diffuse coronary ectasia with slow flows and without hemodynamically significant stenosis. An increasing of matrix metalloproteinases values and a reduction of their tissue inhibitors was showed both during hospitalization and at one month after discharge. This case report emphasizes the close relationship between cocaine abuse, CAE and acute coronary syndromes in patients without hemodynamically significant coronary stenosis. As reported by Satran *et al*, cocaine abuse should be considered an important risk factor for CAE and these patients appear to be at increased risk of angina and acute myocardial infarct. Further studies that can strengthen this hypothesis would be useful to deepen and better analyze this interesting association.

Key words: Coronary artery ectasia; Acute coronary syndromes; Cocaine abuse; Matrix metalloproteinases; Inflammation

Core tip: The pathogenetic role of cocaine abuse in the genesis of coronary artery ectasia (CAE) is still little known and very few data are available in literature. This case report emphasizes the close relationship between cocaine abuse, CAE and acute coronary syndromes in patients without hemodynamically significant coronary stenosis. As reported by Satran *et al*, cocaine abuse should be considered an important risk factor for CAE and appears to be another potential mechanism of

acute coronary syndromes in cocaine users. Further studies that can strengthen this hypothesis would be useful to deepen and better analyze this interesting association.

Dendramis G, Paleologo C, Piraino D, Assennato P. Relationship between coronary artery ectasia, cocaine abuse and acute coronary syndromes. *World J Cardiol* 2016; 8(5): 351-355 Available from: URL: http://www.wjgnet.com/1949-8462/full/v8/i5/351.htm DOI: http://dx.doi.org/10.4330/wjc.v8.i5.351

INTRODUCTION

Coronary artery ectasia (CAE) is a dilation of coronary arteries angiographically defined if the diameter of the artery is at least 1.5 times greater than that of the intact adjacent vascular segment.

Common causes include atherosclerosis, systemic inflammatory disease, systemic vasculitis, genetic connective tissue disorders (Marfan and Ehler-Danlos syndrome) and cocaine abuse.

CAE may have a variable clinical presentation and often represents a coronary angiography finding casually detected or following the occurrence of an atypical chest pain or an acute coronary syndrome. The mechanisms that determine the abnormal dilatation of the vascular lumen are still poorly understood and particularly the overexpression of matrix metalloproteinases (MMPs) has been associated with an excessive expansive arterial remodeling[1].

CASE REPORT

A 31-year-old caucasian man, cocaine user without other cardiovascular risk factors, was admitted to our department for typical acute chest pain started while he was climbing stairs.

At cardiac examination we found a 1/6 Levine systolic murmur at the precordium and blood pressure was 180/90 mmHg. The ECG showed a sinus rhythm with 91 bpm, ST segment depression with T negative waves from V2 to V5 and a corrected QT interval of 493 msec (Figure 1).

Leukocytosis with increasing of inflammatory markers (C-reactive protein 3.2 mg/dL) and positive Troponin I (0.3 ng/mL) were present. Transthoracic echocardiogram showed left ventricular concentric hypertrophy, ejection fraction of 60%, low mitral regurgitation and altered relaxation mitral inflow pattern.

For the persistence of typical chest pain and the high pretest probability of coronary artery disease (CAD) due to cocaine abuse, we choose an invasive diagnostic approach. Percutaneous coronary angiography (performed with 6F diagnostic catheters and right femoral artery access) showed diffuse coronary ectasia without hemodynamically significant stenosis and coronary slow flow with Timi Frame Count score of 2 and a myocardial blush grade 2 (Figure 2). For left coronary artery contrast was injected with a flow rate of 4 mL/s and a volume of 8 mL; for right coronary artery contrast was injected with a flow rate of 3 mL/s and a volume of 6 mL.

To exclude a possible role of a coronary spasm in the genesis of the acute coronary syndrome, an hyperventilation testing and an intracoronary injection of acetylcholine (with incremental doses of 20 and 50 μg into the right coronary artery and of 20, 50, and 100 μg into the left coronary artery over 20 s and with at least a 3-min interval between injections) were performed and were negative. Furthermore, to exclude a thrombophilic diathesis, a thrombophilia testing was also performed and was negative.

For the presence of diffuse CAE MMPs plasma concentrations (MMP-2 and MMP-9) and their tissue inhibitors (TIMP-1 and TIMP-2) were also quantified.

An increasing of MMPs values and a reduction of their tissue inhibitors was showed both during hospitalization (MMP-2: 538.9 ng/mL, normal plasma values 125 ± 30 ng/mL; MMP-9: 53.5 ng/mL, normal plasma values 18 ± 6 ng/mL; TIMP-1: 39.2 ng/mL, normal plasma values 356 ± 110 ng/mL; TIMP-2: 26.3 ng/mL, normal plasma values 105 ± 12 ng/mL) and both at one month after discharge (MMP-2: 492.3 ng/mL, MMP-9: 51.7 ng/mL, TIMP-1: 40.5 ng/mL, TIMP-2: 29.1 ng/mL). Because during acute phenomena these values would be physiologically altered, a double assessment was carried out to ensure that values were not affected by the acute event.

Patient was subjected to pharmacological treatment with acetylsalicylic acid, ACE inhibitors, beta-blockers, spironolactone, and statins. Others possible causes of persistent elevated levels of MMP have not been observed one month after discharge.

DISCUSSION

Cocaine is an alkaloid extracted from the leaf of the Erythroxylon coca bush which blocks the presynaptic reuptake of epinephrine, norepinephrine and dopamine, thereby increasing their postsynaptic concentrations and improving sympathetic activity. Cocaine's principal effects on the cardiovascular system are mediated *via* alpha-adrenergic stimulation with increase in the determinants of myocardial oxygen demand (increasing of heart rate and systemic arterial pressure) and with a concomitant decrease in myocardial oxygen supply caused by vasoconstriction of the epicardial coronary arteries.

Cocaine induced chest pain is a common presentation in emergency departments and premature coronary atherosclerosis with obstructive coronary artery disease often has been seen in young cocaine abusers. Focal occlusive vasospasm, diffuse coronary vasoconstriction, endothelial dysfunction and coronary thrombosis may be responsible for cocaine induced myocardial infarct in patients with normal coronary

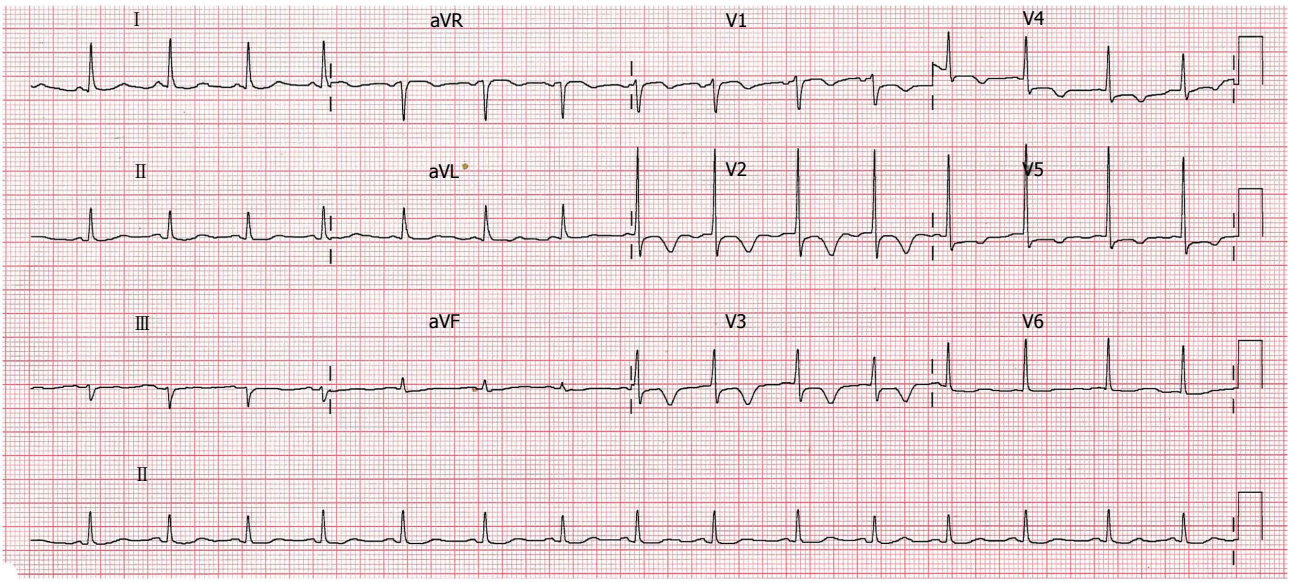

Figure 1 Electrocardiogram at the admission.

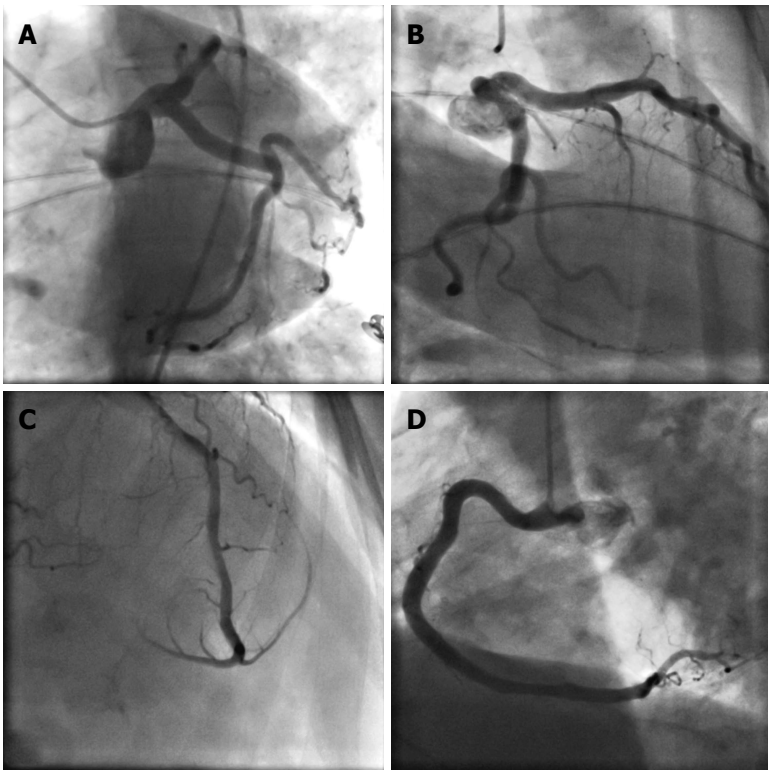

Figure 2 Coronary angiography performed with 6F diagnostic catheters showing ectasia of left anterior descending artery and circumflex coronary artery with maximum diameter of 5.8 mm (A and B), ectasia of medio-distal tract of left anterior descending artery (C), ectasia of right coronary artery with a maximum diameter of 5.6 mm (D).

arteries and cocaine use is also associated with an activation of platelets, leading to increased platelet adhesiveness and aggregation that play a major role in the development of coronary thrombi[2].

Patients with a history of cocaine abuse have also an increased prevalence of CAE. Potential mechanisms that play a major role in the development of CAE may include direct vascular smooth muscle cells apoptosis with media damage, enhanced monocyte migration and endothelium adhesion with hypersecretion of inflammatory cytokines and MMP overexpression with enzymatic degradation and hyalinization of the extracellular matrix. All this effects may cause "chronic vascular stress" (vascular inflammation, arterial remodeling and coronary ectasia) resulting in coronary slow flow and thrombosis[1].

Su *et al*[3] have demonstrated that cerebral vascular smooth muscle cells can undergo rapid apoptosis in

response to cocaine in a concentration-dependent manner, moreover Fiala *et al*[4] have shown that cocaine in vitro increases the expression of endothelial adhesion molecules, intercellular adhesion molecule-1, vascular cell adhesion molecules-1 and platelet/endothelial cell adhesion molecule-1. Moreover, both *in vitro* and *in vivo*, cocaine increases rolling white blood cell flux, leukocyte-endothelium adhesion and mononuclear cells activation with hypersecretion of inflammatory cytokines and overexpression of MMPs with consequent coronary arterial remodeling and ectasia[3,4].

In Demopoulos *et al*[5] study, history of acute myocardial infarct was reported among 39% of patients with CAE but without CAD and for the authors the presence of ectasic vascular segments would lead to a slow blood flow with greater likelihood of intracoronary thrombosis. In an interesting study Satran *et al*[6] have demonstrated as patients with a history of cocaine abuse have an increased prevalence of CAE. These patients appear also to be at increased risk of acute myocardial infarct (nearly half the patients in the cocaine group had a history of acute MI despite an average age of 43 years). Therefore CAE appears to be another potential mechanism of acute coronary syndromes in cocaine users[6].

It is known that CAE can be also caused by long lasting hypertension, but it is also known that the cocaine abuse may determines CAE[6]. In our case the patient did not reported to suffer from hypertension and his young age and lack of familiarity for hypertension does not support the hypothesis that CAE may be secondary to a possible hypertension. Moreover to exclude this hypothesis and on the basis of the echocardiography view of concentric hypertrophy we also investigated possible causes of secondary hypertension but all the exams resulted negative. No others conditions (except cocaine abuse) were present to exclude other causes of CAE.

The pathogenetic role of cocaine abuse in the genesis of CAE is still little known and very few data are available in literature. As demonstrated by Satran *et al*[6], cocaine abuse should be considered an important risk factor for CAE and furthermore these patients appear to be at increased risk of acute coronary syndromes even without hemodynamically significant coronary stenosis.

This case emphasizes the close relationship between cocaine abuse, CAE and acute coronary syndromes in patients without coronary stenosis but, being only a case report, it is not possible to draw conclusions about this association, although often in our clinical practice we see more and more cases like this. Further studies that can strengthen this hypothesis would be useful to deepen and better analyze this interesting association.

COMMENTS

Case characteristics

The authors describe a case of a 31-year-old male cocaine user and without other cardiovascular risk factors, admitted to the authors' department for typical acute chest pain.

Clinical diagnosis

At cardiac examination the authors found a 1/6 Levine systolic murmur at the precordium, blood pressure was 180/90 mmHg and the electrocardiogram showed sinus rhythm, ST segment depression with T negative waves from V2 to V5.

Differential diagnosis

Cocaine induced chest pain is a common presentation in emergency departments and premature coronary atherosclerosis with obstructive coronary artery disease, focal occlusive vasospasm, diffuse coronary vasoconstriction and coronary artery ectasia (CAE) are clinical conditions to be excluded in these patients.

Laboratory diagnosis

Leukocytosis, increasing of inflammatory markers and positive Troponin I were present; furthermore thrombophilia testing was negative and an increasing of matrix metalloproteinase (MMP) values with a reduction of their tissue inhibitors was showed both during hospitalization and at one month after discharge.

Imaging diagnosis

Coronary angiography showed diffuse coronary ectasia with slow flows and without hemodynamically significant coronary stenosis.

Treatment

Patient was subjected to pharmacological treatment with acetylsalicylic acid, ACE inhibitors, beta-blockers, spironolactone, and statins.

Related reports

A double assessment of MMP values and of their tissue inhibitors was carried out both during hospitalization and at one month after discharge to ensure that values were not affected by the acute event, furthermore others possible causes of persistent elevated levels of MMP have not been observed after discharge.

Experiences and lessons

Cocaine abuse should be considered an important risk factor for CAE and appears to be another potential mechanism of acute coronary syndromes in cocaine users; anyway further studies that can strengthen this hypothesis would be useful to deepen and better analyze this interesting association.

Peer-review

The authors reported the acute coronary syndrome accompanied with CAE in a young male cocaine user. This case report is interesting.

REFERENCES

1 **Dendramis G**, Paleologo C, Lo Presti A, Piraino D, Lo Greco V, Grassedonio E, La Grutta L, Midiri M, Assennato P, Novo S. [Coronary artery ectasia: etiopathogenesis, diagnosis and treatment]. *G Ital Cardiol* (Rome) 2014; **15**: 161-169 [PMID: 24770429 DOI: 10.1714/1463.16165]

2 **Lange RA**, Hillis LD. Cardiovascular complications of cocaine use. *N Engl J Med* 2001; **345**: 351-358 [PMID: 11484693 DOI: 10.1056/NEJM200108023450507]

3 **Su J**, Li J, Li W, Altura BT, Altura BM. Cocaine induces apoptosis in cerebral vascular muscle cells: potential roles in strokes and brain damage. *Eur J Pharmacol* 2003; **482**: 61-66 [PMID: 14660005 DOI: 10.1016/j.ejphar.2003.09.056]

4 **Fiala M**, Gan XH, Zhang L, House SD, Newton T, Graves MC, Shapshak P, Stins M, Kim KS, Witte M, Chang SL. Cocaine enhances monocyte migration across the blood-brain barrier. Cocaine's connection to AIDS dementia and vasculitis? *Adv Exp Med Biol* 1998; **437**: 199-205 [PMID: 9666272 DOI: 10.1007/978-1-4615-5347-2_22]

5 **Demopoulos VP**, Olympios CD, Fakiolas CN, Pissimissis EG, Economides NM, Adamopoulou E, Foussas SG, Cokkinos DV. The natural history of aneurysmal coronary artery disease. *Heart* 1997; **78**: 136-141 [PMID: 9326986 DOI: 10.1136/hrt.78.2.136]

6 **Satran A**, Bart BA, Henry CR, Murad MB, Talukdar S, Satran D, Henry TD. Increased prevalence of coronary artery aneurysms among cocaine users. *Circulation* 2005; **111**: 2424-2429 [PMID: 15883217 DOI: 10.1161/01.CIR.0000165121.50527.DE]

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