Intracoronary and systemic melatonin to patients with acute myocardial infarction: protocol for the IMPACT trial

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ABSTRACT

INTRODUCTION: Ischaemia-reperfusion injury following acute myocardial infarctions (AMI) is an unavoidable consequence of the primary percutaneous coronary intervention (pPCI) procedure. A pivotal mechanism in ischaemia-reperfusion injury is the production of reactive oxygen species following reperfusion. The endogenous hormone, melatonin, works as an antioxidant and could potentially minimise the ischaemia-reperfusion injury. Given intracoronarily, it enables melatonin to work directly at the site of reperfusion. We wish to test if melatonin, as an antioxidant, can minimise the reperfusion injury following pPCI in patients with AMI.

MATERIAL AND METHODS: The IMPACT trial is a multicentre, randomised, double-blinded, placebo-controlled study. We wish to include 2 × 20 patients with ST-elevation myocardial infarctions undergoing pPCI within six hours from symptom onset. The primary end-point is the Myocardial Salvage Index assessed by cardiovascular magnetic resonance imaging on day 4 (± 1) after pPCI. The secondary endpoints are high-sensitivity troponin, creatinekinase myocardial band and clinical events.

CONCLUSION: The aim of the IMPACT trial is to evaluate the effect of melatonin on reperfusion injuries following pPCI. Owing to its relatively non-toxic profile, melatonin is an easily implementable drug in the clinical setting, and melatonin has the potential to reduce morbidity in patients with AMI.

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The World Health Organization predicts that ischaemic heart disease will be the leading cause of death worldwide by the year 2020 [1]. It is well known that infarct size is correlated with the short- and long-term outcome following acute myocardial infarction (AMI) [2]. Despite quick and effective treatment, the 90-day mortality following AMI remains 5% [3].

The most widely used and effective treatment is primary percutaneous coronary intervention (pPCI). Although rapid intervention minimises the time that the myocardium is without oxygen, restoring the blood flow and thereby the oxygen supply is not without consequences. Interrupting the blood supply to an organ leads to tissue death, but the reperfusion in itself is also highly damaging. This damage is known as the ischaemia-reperfusion (IR) injury. Animal studies of AMI suggest that lethal reperfusion injury accounts for up to 50% of the final infarct size in AMI [4]. Reactive oxygen species (ROS) or free radicals play an important role in the pathogenesis of cardiac IR injury. Oxidative stress is a consequence of the inefficient utilisation of molecular oxygen (O_2) by cells [5]. ROS are generated as by-products of cellular respiration and other metabolic processes. They damage cellular macromolecules including DNA, proteins and lipids [6].

Melatonin (N-acetyl-5-methoxytryptamine), an endogenously produced hormone, is synthesised by the pineal gland of vertebrates. Its synthesis and release is stimulated by darkness and inhibited by light. Melatonin is known to protect against oxidative stress in cells, and recent publications present evidence that melatonin has cardio-protective properties [7]. It acts directly as a free radical scavenger of reactive oxygen and nitrogen species (receptor independent) and indirectly by up-regulating antioxidant enzymes and down-regulating pro-oxidant enzymes (receptor dependent) [8] through G-protein coupled receptor subtypes named MT_1 and MT_2 [9]. These potent antioxidant effects make melatonin an interesting compound in the clinical setting.

The intracoronary and systemic melatonin to patients with acute myocardial infarction (IMPACT) trial will test the hypothesis that due to its antioxidative properties melatonin will protect the heart from IR injuries following pPCI and thereby increase myocardial salvage compared with placebo.

MATERIAL AND METHODS

The IMPACT trial is a multicentre, randomised, double-blinded, placebo-controlled study to test the effect of intracoronary and systemic administration of melatonin given to patients with AMI while undergoing pPCI (**Figure 1**). The study protocol is approved by the local eth-

PROTOCOL ARTICLE

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FIGURE 1

Percutaneous coronary intervention procedure. The cardiologist can visualize the patient's coronary arteries in the screen using contrast media.

ical committee (H-3-2010-117) and the Danish Health and Medicines Authority (eudraCT number: 2010-022400-53).

Patient selection

For inclusion and exclusion criteria, see **Table 1**. Patients will be excluded if admitted to the intensive care unit.

Definitions of evaluability

1) Safety: The safety population includes all randomised patients who have received the investigational product.
2) Intention to treat: This population is based on the initial treatment assignment and includes all patients who have been randomised regardless of whether they have received the investigational product or not.
3) Per protocol: This population includes all randomised patients who have been treated according to the protocol and have completed a cardiovascular magnetic resonance imaging (CMR) within 4 (± 1) days after pPCI.

Primary end-point

The primary end-point will be the Myocardial Salvage Index (MSI). It will be assessed by CMR on day 4 (± 1) after pPCI. MSI will be calculated as follows: (area at risk (AAR) – infarct size)/AAR. AAR will be assessed by short tau inversion recovery T2-weighted (T2-STIR) imaging. On T2-STIR sequence, oedematous myocardium appears brighter, and the AAR will be traced manually on short-axis images. In cases of T2-STIR images being of poor diagnostic quality, early gadolinium enhancement (EGE) images will be used for manual tracing of AAR. EGE will be acquired as post-gadolinum contrast steady-state free precession (SSFP) scans.

Infarct size will be measured by inversion recovery gradient echo sequence (late gadolinium enhancement (LGE) imaging).

The LGE imaging will be started about ten minutes after intravenous (IV) administration of 0.2 mmol/kg gadolinium-based contrast. The areas of LGE will be quantified on short-axis images using automatic thresholding by the CMR-analysis software. The amount of microvascular obstruction, which appears as dark areas surrounded by enhanced infarcted myocardium, will be assessed manually from LGE images and will be included in the infarct size.

Left ventricular (LV) volumes, mass and systolic function will be assessed by SSFP imaging. Quantifications will be performed manually in short-axis images. Papillary muscles will be included in the LV cavity volume.

Image acquisition protocol

Standard imaging planes will be planned from localizer (scout) scans. The following images will be acquired: SSFP short-axis images covering the entire LV from the atrioventricular plane to the apex at 8/0 mm (slice thickness/slice gap), SSFP long-axis images in 2-, 3-, and 4-chamber views (8 mm slice thickness). T2-STIR images in short-axis (8/0 mm) will be obtained. Afterwards, gadolinium-based contrast will be given IV. Then, SSFP images will be repeated (EGE imaging). Lastly, LGE short-axis images (8/0 mm) and LGE long-axis images in 2-, 3-, and 4-chamber views (8 mm slice thickness) will be acquired.

Secondary end-points

- Blood samples for assessment of cardiac enzymes (high-sensitivity troponin T (hs-TnT) or high-sensitivity troponin I (hs-TnI)). Hs-TnT/hs-TnI will be measured in a peripheral blood sample preoperatively and 6, 24, 48, 72 and 96 hours after pPCI. Hs-TnT/hs-TnI will be calculated as the area under the curve (AUC).
- Creatinekinase myocardial band, (CK-MB) will be measured in a peripheral blood sample preoperatively and 6, 24, 48, 72 and 96 hours after pPCI.
- Plasma melatonin and markers of oxidative stress: advanced oxidative protein products, malondialdehyde and myeloperoxidase will be collected during hospital admission 24 h post pPCI.
- Clinical events occurring within the first 90 days after pPCI: Sustained ventricular arrhythmias, resuscitation after cardiac arrest, cardiogenic shock, revascularization of a new coronary artery, coronary artery bypass graft, major bleedings, re-infarction, stent thrombosis, cardiac and non-cardiac re-hospitalization, and death.

Study design

Patients who meet the inclusion criteria will be randomised to either melatonin (M-5250, Sigma-Aldrich, St. Louis, Missouri, USA) or placebo (isotonic saline). Patients randomised to melatonin will receive a total dose of 50 mg, 1 mg given as an intracoronary (IC) bolus (10 ml 0.1 mg/ml) and 49 mg (490 ml 0.1 mg/ml) given IV in a peripheral vein. A bolus of either melatonin or placebo will be given through the PCI-guiding catheter within the first 60 seconds after restoring the blood flow to the infarct-related artery. The 490 ml melatonin or placebo will be given over a time period of six hours commencing immediately after pPCI. pPCI will be performed according to the standard clinical practice and at the operator's discretion. Any medication given in relation to the pPCI will be according to the standard guidelines at the trial centres.

Blood samples will be collected before the reperfusion and 6, 24, 48, 72 and 96 hours post-intervention.

On day 4 (± 1) after pPCI, the patients will undergo CMR to assess the primary end-point.

At 90 days post-intervention, the medical journals will be obtained in order to register any clinical events in the 90-day period.

Sample size

Based on previous results [10] that tested the cardioprotective effect of exenatide following coronary occlusion and subsequent reperfusion in patients undergoing pPCI, we assume that the average salvage index measured by CMR will be 0.62 with a standard deviation of 0.16. With a type 1 error at 5% and a type 2 error at 20%, and the smallest effect that would be important to detect at 25%, it is possible by CMR to detect a difference in infarct size (MSI) between the two groups by including 17 patients into each group. We have decided to include 20 patients into each group.

Statistical analysis

Regarding the primary end-point, the following data will be reported for the two groups in the CMR analysis: MSI defined as (AAR-infarct size)/AAR, myocardial salvage (AAR-infarct size), infarct size (absolute and % of AAR and % of LV), AAR (absolute and % of LV), LV dimensions (mass in diastole, end-diastolic volume, end-systolic volume), left ventricular function (stroke volume and ejection fraction) and the amount of microvascular obstruction (absolute and % of AAR and % of LV).

Regarding the primary endpoint MSI, normality of the data will be tested using the Kolmogorov-Smirnov test, and parametric or non-parametric statistics will be used accordingly. If data are parametric, we will test for statistical significance using unpaired t test. Data will be described using mean and standard deviation. If data are non-parametric, we will test for statistical significance for two independent samples using the Mann-Whitney U test. Data will be described using medians and ranges. Categorical variables will be analysed using either Fisher's exact test or the χ^2-test. Generally, a two-sided p value of < 0.05 will be considered statistically significant. Data will be analysed using SPSS/PC+ package 20.0 (SPSS Inc., Chicago, IL, USA).

Secondary end-points

Data will be analysed according to the same principles as described for the primary endpoint.

Randomisation and blinding

The randomisation list is generated by the hospital pharmacy, Herlev, Denmark, at which the melatonin is also tested for sterility and packaged in glass bottles. There are two sealed envelopes containing the randomisation code for each patient; one used for randomisation by the assisting nurse at the time of pPCI; the other to be kept with the patients case report form to double-check allocation when the study is terminated. The assisting nurse will also be responsible for mixing the melatonin powder with isotonic saline if the patient is randomised to active treatment. Thereby the investigator, the op-

TABLE 1

Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Adults > 18 years of age	Known prior myocardial infarction
pPCI initiated within 6 h from symptom onset	Prehospital thrombolysis
Having an electrocardiogram indicative of an acute ST-elevation myocardial infarction showing: ≥ 0.2 mV in V2 or V3 and/or ≥ 0.1 mV in the other leads for men < 40 years: ≥ 0.25 mV in V2-V3	ASA class ≥ 4
	> 1 artery with coronary stenosis that requires treatment
	Known history of renal failure (GFR < 60 ml/min./1.73 m^2 p-cratinin > 200 µmol/l)
Occlusion of a large (> 2 mm) infarct-related coronary artery with TIMI 0-1	Known history of autoimmune diseases (systemic lupus erythematosus, mulitple sclerosis, rheumatoid arthritis, type 1 diabetes mellitus)
Being willing and able to provide informed consent after written and oral information	Servere concurrent illness with reduced short-term prognosis (e.g. terminal cancer, terminal AIDS, severe infection)
	Atrial fibrillation before or after pPCI
	Pregnancy
	Breastfeeding
	Fertile woman < 12 months since menopause or nonsterilised)
	Cardiogenic shock
	BMI > 40 kg/m^2
	Contraindications for CMR (pacemaker, parts of metal in the body and claustrophobia)

ASA class = The American Society of Anesthesiologists Physical Status classification; BMI = body mass index; CMR = cardiovascular magnetic resonance imaging; GFR = glomerular filtration rate; pPCI = primary percutaneous coronary intervention; TIMI = thrombolysis In myocardial infarction.

erating cardiologist and the patient will be blinded throughout the study. The analysis of the CMR will also be kept blinded, and the overall statistical analyses will be performed without knowing which treatment group the patients belong to. The allocation code will be revealed only after all analyses are completed.

The IMPACT trial has a safety committee. The committee consists of three senior consultant doctors specialising in cardiology, anaesthesiology and surgery. The safety committee is not in any way involved in the trial, but will be contacted if a study subject experiences a serious adverse reaction or a serious adverse event. If they find it plausible that the serious adverse reaction/event is directly related to the trial, they can have the subject unblinded, and ultimately the trial might be terminated.

Trial registration: www.clinicaltrials.gov, Clinical trials identifier: NCT01172171.

DISCUSSION

One of the known mechanisms involved in IR injury is the massive generation of ROS [11]. Melatonin, one of the most potent endogenous antioxidants [12], is highly lipid-soluble, but also soluble in aqueous solutions. Thus, melatonin readily enters all cells and subcellular compartments and cross morphophysiologic barriers [13] and exhibits its antioxidant functions.

Mitochondria are known to be intimately involved in the processes that lead to cell death following reperfusion [14]. Permeability transition in the mitochondria is caused by the opening of the mitochondrial permeability transition pore (mPTP) [15]. Massive mPTP opening results in mitochondrial depolarisation, swelling and rupture of the external mitochondrial membrane and efflux of cytochrome c and other proapoptotic factors that may lead to either apoptosis or necrosis. It has been suggested that mPTP remains closed during the ischaemic period, and at reperfusion there is an influx of Ca^{2+} into the mitochondria resulting in a burst of ROS production. Identification of agents that can protect the heart from the damaging effects of mPTP is of considerable importance in attenuating IR injuries [15]. Melatonin directly inhibits the mPTP [16], and this makes melatonin an apparent choice when considering possible treatments with regard to IR injuries.

Melatonin is relatively non-toxic [17]. Doses of 1,000 mg daily for one month have been given to humans and the only reported adverse effect was drowsiness [17]. In a recent systematic review, the most frequently reported side effects were headache, dizziness, nausea and drowsiness, but with frequencies comparable to placebo [18]. We have chosen the 50 mg melatonin dose as we have previously shown (unpublished data) that this dose significantly reduced the cardiac morbidity and markers of myocardial injury after major vascular surgery.

With regard to our primary outcome, we chose MSI assessed by CMR. CMR is uniquely able to integrate in a single examination an accurate quantitative assessment of LV function, structural abnormalities of the myocardial tissue including oedema, infarct size and myocardial salvage as well as its microvascular status. Therefore, CMR has a potential as the main diagnostic tool in AMI by providing information on the stage, degree and extent of reversible and irreversible myocardial injury [19, 20]. Thus, we find that CMR is the best available method for the determination of our primary outcome.

CONCLUSION

In conclusion, we intend to examine the effect of melatonin in patients with AMI undergoing pPCI. If the results are positive, this simple treatment may be included for pPCI with revascularisation in a larger scaled study, and it would be of interest to examine the effect of melatonin given as a prehospital treatment to further reduce the IR injury. The diversity of melatonin's physiological functions and treatment effects are continuously being investigated in both animal and human studies. To date, the effect of IC melatonin in patients with AMI has not yet been studied.

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LITERATURE

1. Lopez AD, Murray CC. The global burden of disease, 1990-2020. Nat Med 1998;4:1241-3.
2. Thompson PL, Fletcher EE, Katavatis V. Enzymatic indices of myocardial necrosis: influence on short- and long-term prognosis after myocardial infarction. Circulation 1979;59:113-9.
3. Stebbins A, Mehta RH, Armstrong PW et al. A model for predicting mortality in acute ST-segment elevation myocardial infarction treated with

primary percutaneous coronary intervention: results from the Assessment of Pexelizumab in Acute Myocardial Infarction Trial. Circ Cardiovasc Interv 2010;3:414-22.
4. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. N Engl J Med 2007;357:1121-35.
5. Reiter RJ, Tan DX, Gitto E et al. Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage. Pol J Pharmacol 2004;56:159-70.
6. Kozina LS, Arutjunyan AV, Khavinson VK. Antioxidant properties of geroprotective peptides of the pineal gland. Arch Gerontol Geriatr 2007;44(suppl 1):213-6.
7. Tengattini S, Reiter RJ, Tan DX et al. Cardiovascular diseases: protective effects of melatonin. J Pineal Res 2008;44:16-25.
8. Hardeland R. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. Endocrine 2005;27:119-30.
9. Dubocovich ML, Markowska M. Functional MT1 and MT2 melatonin receptors in mammals. Endocrine 2005;27:101-10.
10. Lonborg J, Vejlstrup N, Kelbaek H et al. Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. Eur Heart J 2012;33:1491-9.
11. Turer AT, Hill JA. Pathogenesis of myocardial ischemia-reperfusion injury and rationale for therapy. Am J Cardiol 2010;106:360-8.
12. Reiter RJ. The role of the neurohormone melatonin as a buffer against macromolecular oxidative damage. Neurochem Int 1995;27:453-60.
13. Karbownik M, Reiter RJ. Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. Proc Soc Exp Biol Med 2000;225:9-22.
14. Rodriguez-Sinovas A, Abdallah Y, Piper HM et al. Reperfusion injury as a therapeutic challenge in patients with acute myocardial infarction. Heart Fail Rev 2007;12:207-16.
15. Halestrap AP, Clarke SJ, Javadov SA. Mitochondrial permeability transition pore opening during myocardial reperfusion – a target for cardioprotection. Cardiovasc Res 2004;61:372-85.
16. Andrabi SA, Sayeed I, Siemen D et al. Direct inhibition of the mitochondrial permeability transition pore: a possible mechanism responsible for anti-apoptotic effects of melatonin. FASEB J 2004;18:869-71.
17. Nordlund JJ, Lerner AB. The effects of oral melatonin on skin color and on the release of pituitary hormones. J Clin Endocrinol Metab 1977;45:768-74.
18. Buscemi N, Vandermeer B, Hooton N et al. Efficacy and safety of exogenous melatonin for secondary sleep disorders and sleep disorders accompanying sleep restriction: meta-analysis. BMJ 2006;332:385-93.
19. Friedrich MG. Tissue characterization of acute myocardial infarction and myocarditis by cardiac magnetic resonance. JACC Cardiovasc Imaging 2008;1:652-62.
20. Friedrich MG. Myocardial edema – a new clinical entity? Nat Rev Cardiol 2010;7:292-6.