L-triiodothyronine (T3) for use in limiting microvascular obstruction

The present invention concerns a novel treatment and treatment regimen after a successful Primary Percutaneous Coronary Intervention (PPCI), in the course of an acute ST-segment–elevation myocardial infarction (STEMI), with a view to limit or prevent the occurrence of Microvascular Obstruction (MVO).

STEMI is initiated by occlusion of a large epicardial coronary artery due to atherosclerotic disease. A successful PPCI restores blood flow in the occluded coronary artery. However, during the reperfusion an obstruction can occur at the level of small vessels, a phenomenon known as MVO, and thus blood flow may not be restored in all myocardium despite successful PPCI on large vessels. MVO is a prevailing and common complication after PPCI for STEMI patients and is associated with larger infarct size, poor recovery of ventricular function and reduced survival.

Acute myocardial infarction is a common cause of patients increased mortality and morbidity. Prompt restoration of blood flow in the occluded epicardial coronary artery by primary percutaneous coronary intervention (PPCI), after an acute ST-segment–elevation myocardial infarction (STEMI), is currently the most effective therapy for reducing myocardial infarct size and preserving left ventricular systolic function. However, mortality and morbidity remain significant. Current evidence suggests that this is mainly attributed to the development of microvascular obstruction (MVO), and is associated with adverse left ventricular remodeling and worse clinical outcomes. The gold standard for diagnosis of MVO is contrast-enhanced cardiac magnetic resonance within 14 days after STEMI. MVO can be quantified in each patient as the percentage of left ventricular (LV) myocardium that is not adequately perfused despite restoration of blood flow in the occluded large coronary artery. MVO can be also assessed with cine coronary angiography, incomplete resolution of ST segment elevation measured on electrocardiography and myocardial contrast

echocardiography. To date, there is no effective therapy to prevent or minimize the burden of MVO and is currently considered irreversible.

The phenomenon of MVO refers to the inability to reperfuse a previously ischemic myocardium in the presence of a patent epicardial coronary artery. MVO occurs in almost half of STEMI patients after PPCI and is associated with worse outcomes, independent of the infarct size (J Thorac Dis. 2018 Mar;10(3):1343-1346). In a meta-analysis of 1025 STEMI patients reperfused by PPCI and with a CMR performed within the first week, MVO was associated with the occurrence of a composite of cardiac death, congestive heart failure, and myocardial reinfarction with a hazard ratio of 3.74, whereas Myocardial Infarction (MI) size was not, in a multivariate Cox regression analysis, after adjusting for confounders. In a more recent meta-analysis using individual patient data from seven randomized primary PCI trials (n = 1688 patients), MVO was assessed within 7 days after reperfusion by CMR using late gadolinium enhancement imaging (Eur Heart J. 2017 Dec 14;38(47):3502-3510). Patients were analyzed using as a cut-off point for MVO the value of 0.47%, which was the median value (50% of patients were below and 50% were above this value). Increased hazard ratio for the 1-year composite endpoint of all-cause mortality or heart failure hospitalization was found in patients with large extent of MVO (above the median of 0.47%). This effect was independent from infarct size, age, gender, smoking status and the existence of hypertension, hyperlipidemia and diabetes.

Despite having a patent epicardial coronary artery post-PPCI, those patients with MVO have areas of ongoing hypoperfusion at the microcirculation level. Therapeutic approaches that could theoretically reduce MVO by achieving patency of the microcirculation, have failed. Remarkably, important advances in PCI techniques (such as aspiration thrombectomy or deferred stenting strategy) have had limited impact on the incidence of MVO and related outcomes [*J Thorac Dis* 2018;10(3):1343-1346]. Recently, high-dose intracoronary adenosine and sodium nitroprusside failed to reduce MVO and MI size in a cohort of 247 reperfused STEMI patients [*Eur Heart J*. 2016;37:1910–1919.] Some experimental studies

demonstrated that the initiation of therapeutic hypothermia minutes after restoring flow in a proximally-occluded coronary artery significantly reduced the incidence of no-reflow, but did not affect infarct size.

In summary, there are no effective treatments for MVO in STEMI patients available. Thus, research validating novel therapies in this arena is urgently needed. In this context, the present invention refers to a novel effect of L-triiodothyronine (T3) on limiting microvascular obstruction after PPCI in STEMI patients.
However, to date administration of thyroid hormones (TH) in all possible situations involving acute myocardial infarction is avoided due to long held belief that TH may be detrimental for the ischemic myocardium. A strong prejudice exists among clinicians to consider even the restoration of TH levels to normal in hypothyroid patients with acute myocardial infarction. In fact, the current belief is that the use of TH in this setting can increase heart rate which results in enhanced oxygen consumption and energy expenditure and thus, could result in worsening of myocardial injury and infarct size. Furthermore, high levels of TH have been related to increased incidence of arrhythmias which is one of the causes of early mortality in these patients. In this regard, early clinical studies, such as the Coronary Drug Project in 1970s, have shown that long-term administration of high doses D-thyroxine in patients after acute myocardial infarction resulted in increased mortality. These data have resulted in very cautious use of TH in patients with coronary artery disease (CAD) in general. However, strong epidemiological data showing that stress induced low T3 is associated with worse outcomes in patients with CAD, has revived research interest in the field. According to this evidence, several experimental studies were conducted to investigate the efficacy of long-term TH replacement therapy in restoring cardiac function in animals with experimental myocardial infarction. Treatment with near physiological doses of TH was shown to favorably remodel the postischemic myocardium with favorable changes in myosin heavy chain (MHC) expression pattern (less β-MHC and increased α-MHC), ellipsoid reshaping and improved left ventricular ejection fraction. Interestingly, in a mouse model of myocardial infarction, TH replacement therapy restored recovery of function while

long-term treatment with high doses of TH resulted in increased mortality (Ann Transl Med. 2018 Jun;6(12):254).

Documents US2011/0142947A1 and US2005/0059574A1 refer to T3 compositions suitable for use in cardiac arrest, cardiac arrest with cardiac electrical standstill, cardiogenic shock and acute heart failure. These pathological entities are distinct from myocardial infarction or myocardial ischemia and reperfusion. In fact, a small portion of patients with STEMI (5-10%) may present with cardiac arrest, cardiogenic shock and acute heart failure.

Documents WO 95/00135 and Mdzinarishvili A. in *Drug Deliv. and Transl. Res.* (2013) 3:309–317, refer to the use of T3 in central nervous system ischemia and ischemic brain stroke. These pathological entities are entirely distinct from MI or myocardial ischemia and reperfusion and in this context are completely out of the scope of the current invention.

Document WO 02/051403 refers to the value of measuring T3 levels in blood as a biomarker to evaluate prognosis in patients with myocardial infarction (indicator for mortality). However, especially Example 1 refers to reverse T3 (rT3) which is a different molecule compared to T3, in particular, the missing de-iodinated iodine is from the inner ring of the thyroxine molecule compared with outer ring on T3. Due to this fact reverse T3 is inactive.

The potential of TH replacement therapy in patients with acute myocardial infarction (AMI) has been tested in a recent phase II clinical trial (THIRST study – EudraCT: 2009-010869-23). In this randomized controlled trial of oral T3 in 37 patients with STEMI and low serum FT3 levels, T3 replacement therapy for 6 months was shown to be safe and effective in reducing regional cardiac dysfunction. Left ventricular ejection fraction and myocardial infarct size, as assessed by cardiac MRI, did not show any benefit. In this trial, T3 was commenced 72 h after the onset of STEMI, and the preparation was administered three times per day (maximum dosage 15 $\mu g/m^2$/day). This small trial provides evidence that normalization of serum T3 levels in patients with STEMI who have low circulating T3 levels is safe, if T3 therapy is commenced a few days after the acute event.

The potential effect of early administration of high dose TH (acute treatment) after an index event has been also investigated in experimental models of ischemia-reperfusion (I/R) using isolated rat heart preparations. Thus, high dose T3 (triiodothyronine) administration at the onset of reperfusion resulted in enhanced post-ischemic recovery of function and less myocardial injury as indicated by apoptosis. In this ex vivo experimental setting, T4 (L-thyroxine) was shown to have no effect on myocardial injury (Heart Fail Rev. 2015 May;20(3):273-82.). Based on this evidence, a phase II study (Thy-REPAIR – EudraCT: 2016-000631-40) was designed to translate these results in clinical practice. Thy-REPAIR explores the effects of high-dose T3 treatment intravenously starting immediately after reperfusion and continuing for 48 hours in patients with anterior or anterolateral STEMI undergoing primary PCI. This is a double-blind randomized trial. The primary end point of this study is recovery of myocardial function after 6 months of the index event as this is assessed by Cardiac Magnetic Resonance (CMR) at discharge and at 6 months after acute myocardial infarction. This study also explores the potential effects of T3 treatment on infarct size and cardiac remodeling by assessing changes in LV volumes and geometry.

In the course of this study certain observations were made that led to the present invention.

The invention in particular concerns L-triiodothyronine for use in the treatment as set forth in the appending claims. In the broader sense, the present invention concerns L-triiodothyronine for use in the treatment of microvascular obstruction. Treatment of MVO in this context relates to the lessening of microvascular obstruction in comparison to patients which were not supplied with T3. MVO may be determined on the basis of both Cardiac Magnetic Resonance (CMR) imaging, being performed 7-12 days following primary percutaneous coronary intervention (PPCI) and restoration of ST elevation in electrocardiogram (ECG). Infarct size and ejection fraction was measured by CMR. Infarct size was also assessed enzymatically by measuring levels of troponin in blood.

The present invention in particular concerns a medicament comprising triiodothyronine (T3) administered to ST-elevation myocardial infarction patients that undergo primary percutaneous coronary intervention (PPCI).

Disclosed herein is the use of L-triiodothyronine (T3) in the manufacture of a medicament for the treatment of microvascular obstruction (MVO) in ST-segment-elevation myocardial infarction (STEMI) patients, wherein said treatment is commenced after primary percutaneous coronary intervention (PPCI).

The present invention in particular concerns use of a T3 solution that is administered intravenously as a solution with a concentration of T3 in the range of 5 to 20 µg/ml, in particular 10 µg/ml.

Typically, the treatment is commenced with the administration of a relative high dose after reperfusion. This bolus may be in the range of 0.7 µg/kg to 0.9 µg/kg. Thus, with a T3 solution comprising 10 µg L-triiodothyronine in 1 ml solution, patients may receive a bolus administration over 2-3 min of in between 4.0 and 8.0 ml.

This bolus may be administered intravenously.

Subsequent to the high-dose bolus, the patients receive a continued infusion over 24-72 hours. Typically, the patients receive a continuous injection of T3 in the range of 0.1 to 0.2 µg/kg/h.

These doses are considered much higher than in the normal treatment of patients with non-adequate thyroid functions (e.g. patients with hypothyroidism or myxedema). Accordingly, and in the course of a treatment over about 48 h, a patient in the weight range of 60 kg to 100 kg, may receive in total 350 to 600 µg T3. This

dose is 8-12 times higher compared to the dose used in the Thirst trial to restore T3 levels to normal range.

DESCRIPTION OF THE DRAWINGS

Figure 1: Levels of triiodothyronine in serum during hospitalization in patients after acute myocardial infarction treated with placebo and T3

Figure 2: Heart rate assessed during hospitalization in STEMI patients treated with placebo and T3

Figure 3: Extent of scar as percentage of the Left ventricle (LV) assessed by Cardiac Magnetic Resonance at discharge in STEMI patients treated with placebo and T3

Figure 4: Mean MVO assessed by Cardiac Magnetic Resonance at discharge in STEMI patients treated with placebo and T3

Figure 5: Percentage of patients with large extent of MVO in placebo and T3 treated group. T3 significantly reduces the percent of patients with large extent of MVO

Figure 6: Electrocardiogram of a patient with STEMI on admission (A) and after successful PPCI (B). Note the ST elevation in A (blue arrows) showing the existence of myocardial ischemia and the resolution of ST elevation (red arrows) in B indicating the restoration of blood flow. In patients with large extent of MVO, ST elevation in ECG after PPCI is not restored indicating persistent myocardial ischemia

Figure 7: Resolution of ST elevation (%) in electrocardiograms performed on admission and after PPCI in placebo and T3 groups

Figure 8: Troponin levels over time in STEMI patients treated with placebo and T3

Figure 9: Correlation of cardiac function (ejection fraction at 6 months) and infarct size measured by CMR in placebo group. There is a strong inverse relationship in accordance with previous studies

Figure 10: Correlation of cardiac function (ejection fraction at 6 months) and infarct size measured by CMR in T3 treated group. Cardiac function is not related to the extent of injury indicating a different pathophysiological process

Figure 11: CMR analysis shows no difference in infarct size (extent of injury) between placebo and T3 group

Figure 12: Left ventricular ejection fraction assessed by echocardiography at different time points in STEMI patients treated with placebo and T3

Figure 13: Left ventricular ejection fraction and left ventricular end-diastolic (LVEDVi) and end-systolic (LVEDVi) volumes assessed by Cardiac Magnetic Resonance at discharge in STEMI patients treated with placebo and T3

Figure 14: Left ventricular ejection fraction and left ventricular end-diastolic (LVEDVi) and end-systolic (LVEDVi) volumes assessed by Cardiac

Magnetic Resonance at 6 months in STEMI patients treated with placebo and T3

A practitioner may consult the following explanation and tables, which show a dosage schedule of T3 administration according to the patient's weight.

Table 1: Dosage schedules of T3 Solution for injection 10 µg/ml according to patient's weight

Patient's weight	**Bolus administration over 2-3 min**	**Continuous infusion per 24 hours (pump rate 10.4 mL/h)**
62Kg (min)	5.0 ml (50µg T3)	17ml (170µg T3) in 233 ml NaCl 0.9%
66Kg	5.5 ml (55µg T3)	18ml (180µg T3) in 232 ml NaCl 0.9%
70Kg	5.5 ml (55µg T3)	19ml (190µg T3) in 231 ml NaCl 0.9%
74Kg	6 ml (60µg T3)	20ml (200µg T3) in 230 ml NaCl 0.9%
77Kg	6 ml (60µg T3)	21ml (210µg T3) in 229 ml NaCl 0.9%
81Kg	6.5 ml (65µg T3)	22ml (220µg T3) in 228 ml NaCl 0.9%
85Kg	7.0 ml (70µg T3)	23ml (230µg T3) in 227 ml NaCl 0.9%
89Kg	7.0 ml (70µg T3)	24ml (240µg T3) in 226 ml NaCl 0.9%
92Kg	7.5 ml (75µg T3)	25ml (250µg T3) in 225 ml NaCl 0.9%
>95Kg (max)	7.5 ml (75µg T3)	26ml (260µg T3) in 224 ml NaCl 0.9%

As a further example, the practitioner may consider the following.

For a patient of 77 Kg of weight, a dose of 6 ml of T3 Solution for injection 10 µg/ml (total of 60 µg T3) is administered intravenously as a bolus over 2-3 min, upon the beginning of reperfusion during primary PCI. Subsequently and for the next 24 hours, the patient receives a solution comprising of 21 ml of T3 Solution for injection 10 µg/ml (total of 210 µg of T3) diluted in 229 ml of NaCl 0.9%, which are

administered in the aid of a pump at a steady flow rate of 10.4 ml/h. The last step repeated for another 24 hours, thus the total amount of T3 administered to the patient from the beginning of the treatment is 480 µg. Table 2 summarizes the total amount of T3 administered to each patient from the beginning of the treatment.

Table 2: Total amount of T3 administered to each patient after treatment for 48 h

Patient's weight	**Total Dose of T3 during the 48 hours period of administration**
62Kg (min)	390µg
66Kg	415µg
70Kg	435µg
74Kg	460µg
77Kg	480µg
81Kg	505µg
85Kg	530µg
89Kg	550µg
92Kg	575µg
>95Kg (maximum)	575µg

Apparently, the present invention is based on the surprising observation that with the dosage regimen as considered in the present invention (high-dose of T3 above any consideration of a thyroid replacement therapy) the extent of microvascular obstruction (MVO) is lowered. MVO after reperfusion is now recognized as an important prognostic factor of survival in these patients independent from infarct size. Thus, any reduction of MVO can reduce the mortality rate of STEMI patients, which undergo treatment with PCI.

Triiodothyronine in the study is used in the form of a T3® Solution for injection 10 µg/ml that contains 150 µg of L-triiodothyronine in a total volume of 15 ml per vial. The medicament is a clear, colorless solution containing the active substance Liothyronine sodium and other ingredients including dextran 70, NaOH 1 N and water for injection. Liothyronine sodium is synthesized in vitro. The medicament could also be supplied in the lyophilized form and reconstituted with water for injection or saline immediately prior to use.

Example 1

No	Name of ingredient(s)	Quantity / 1ml
	Active ingredient:	
1.	Liothyronine sodium	10.0 µg
	Other ingredient (s):	
1.	Dextran 70	60.0 mg
2.	NaOH 1 N	q.s. pH 10
3.	Water for Injections	qs 1.0

The dose administered is 0.8 µg/kg intravenously bolus starting immediately after successful opening of the culprit vessel has been established in the hemodynamic lab and followed by an infusion of 0.113 µg/kg/h intravenously for 48 hours. Administration of the medicament increases significantly the levels of T3 in serum at 24 and 48 hours (5.5 (0.5) ng/ml and 4.5 (0.5) ng/ml respectively compared to 0.79 (0.06) and 0.76 (0.06) ng/ml for the placebo group, $p<0.01$). This treatment increases T3 levels in serum far above normal range for 72 hours and allows return to normal at discharge (7^{th} day) (Figure 1).

Administration of high dose T3 resulted in a non-significant increase in heart rate during the first 3 days (Figure 2). Despite the possible increase in oxygen consumption, no increase in infarct size (IS) is found with T3 in contrast to the current belief (Figure 3).

Beyond our expectations, an important and surprising finding of the present study was revealed from the routine analysis of contrast-enhanced cardiac magnetic resonance which involves the determination of mean microvascular obstruction and the incidence of microvascular obstruction in these patients. Actually, cardiac magnetic resonance with gadolinium represents the gold-standard method for detection of MVO in patients with STEMI. MVO can be quantified in each patient as the percentage of left ventricular (LV) myocardium that is not adequately perfused despite restoration of blood flow in the occluded large coronary artery.

In fact, the extent of mean microvascular obstruction was less in the T3 treated STEMI patients based on CMR data at discharge (7-12 days after the event) (Figure 4).

The incidence of MVO was assessed using the value of 0.47% as a cut-off point for patients with large and small extent of MVO as previously described. This analysis was additionally performed and provides a strong evidence about the T3 effect on MVO. In the placebo group (n=9), a large extent of MVO was found in 56% of patients consistent with previous published reports in the literature. Surprisingly, in the T3 treated group (n=12), a large extent of MVO was found only in 25% of patients, p=0.05 (Figure 5).

These unexpected findings concerning MVO were also supported by other measurements. Changes in ST elevation in electrocardiograms before and after PPCI measured as ST restoration (%) are closely correlated with the extent of MVO

(Figure 6). In fact, recent evidence suggests that incomplete resolution of ST-segment elevation after PPCI in patients with STEMI reflects the presence of microvascular and left ventricular dysfunction, especially in patients with left anterior descending artery (LAD) infarction.

The Mean ST resolution after PPCI was found higher in T3 than in the placebo group (Figure 7). The mean ST resolution was measured 34% in the placebo group compared to 54% in the T3 treated group ($p=0.05$). These measurements provide strong evidence of the effect of T3 on MVO.

Troponin levels in blood over time are mainly a measure of myocardial injury. According to the present invention, troponin levels are similar between T3 and placebo groups indicating a similar infarct size in accordance with CMR data. Thus, it seems that the effect of T3 on MVO is independent from infarct size. (Figure 8).

Accordingly, T3 treatment was associated with improved cardiac function at discharge and at 6 months. This effect, as our data show, is rather attributed to the effect of T3 on MVO than to infarct size. According to Figure 9, the functional recovery at 6 months in the placebo group is largely dependent on infarct size. Thus, a higher infarct size results in lower functional recovery at 6 months. This is consistent with prior art.

Surprisingly, it was found that in T3 group (Figure 10), the recovery of function was independent of the myocardial injury indicating other novel effects of T3 on the ischemic myocardium (i.e. effects on MVO). No experimental or clinical study so far has described this particular T3 effect on myocardial healing (repair) after ischemia/reperfusion injury.

Interestingly it was found that, although CMR clearly indicate that infarct size was similar between the placebo and T3 group (see Figure 11), echocardiographic data show an improvement of LV ejection fraction in T3 treated STEMI patients early and late after the index event (see Figure 12).

Additional evidence for the improved myocardial function due to T3 treatment was demonstrated by left ventricular ejection fraction (EF%) and left ventricular end-diastolic (LVEDVi) and end-systolic (LVEDVi) volumes, as these assessed by CMR for T3 group and placebo group at discharge (Figure 13).

Similarly, improvement of cardiac function in T3 treated patients persisted at 6 months (Figure 14).

Claims

1. Use of L-triiodothyronine (T3) in the manufacture of a medicament for the treatment of microvascular obstruction (MVO) in ST-segment-elevation myocardial infarction (STEMI) patients, wherein said treatment is commenced after primary percutaneous coronary intervention (PPCI).

2. Use according to claim 1, wherein the MVO is measurable by cardiac magnetic resonance.

3. Use according to claim 1 or claim 2, wherein the medicament is formulated for administration intravenously as a solution with a concentration of T3 in the range of 5 to 20 µg/ml.

4. Use according to claim 3, wherein said patients receive an initial bolus of 0.7 to 0.9 µg T3 per kg weight of the patient.

5. Use according to claim 3 or 4, wherein said patients subsequent to the bolus receive a continuous injection of 0.1 to 0.2 µg/kg/h.

6. Use according to claim 4 or 5, wherein said patients receive in total 350 to 600 µg T3 over about 48 hours.