Cardiac troponin I (cTnI) has been proposed for use in early assessment of reperfusion therapy (1–4). However, commercial cTnI assays may react differently depending on the circulating forms of cTnI (5). Numerous reports have focused on the existence of troponin I complexes with the two other troponin components in the bloodstream of patients with myocardial infarction (6,7). Independent of cTnI proteolysis occurring in the bloodstream related to the existence of covalent complexes of cTnI with cardiac troponin C (cTnI-cTnC) and T (cTnT-cTnI-cTnC) (8), cTnI degradation products also occur within human myocardium (9). To our knowledge however, no report has identified the cTnI forms released in patients with acute myocardial infarction (AMI) undergoing reperfusion therapy. Our purpose was to study cTnI release in patients undergoing successful (TIMI 3) reperfusion by primary percutaneous transluminal coronary angioplasty (PTCA) with stenting to ascertain and visualize rapid restoration of the coronary flow.

We first established the release characteristics of cTnI and creatine kinase MB (CK-MB) in 11 AMI patients [9 males and 2 females; age range, 51–80 years; 4 with an occluded right coronary artery (RCA), and 7 with an occluded left anterior descending artery (LAD)] undergoing PTCA who presented within 6 h after onset of chest pain (2–6 h). All patients received standardized adjunctive therapy consisting in abciximab, heparin, clopidogrel, and aspirin. Plasma (heparin) cTnI (10) and CK-MB concentrations were measured on the DPC Immulite [upper reference limit (URLs): cTnI, 1 µg/L (ROC curve); CK-MB, 4.8 µg/L (99th percentile)]. Samples were taken before PTCA (0 min) and 30, 60, 90, and 120 min afterward. As shown in Table 1, the cTnI concentration before PTCA was below the URL in all of the studied patients. The release profiles of cTnI and CK-MB linearly increased during this period: 

\[
y = 1.14x - 2.91 \mu g/L \quad (r = 0.998) \quad \text{and} \quad y = 2.37x + 11.78 \mu g/L \quad (r = 0.999), \text{respectively.}
\]

We attempted to identify cTnI forms (free, complexed, and degraded cTnI) released in four different patients by use of fast protein liquid chromatography (FPLC; Pharmacia) with prepacked Superdex 200 HR 10/30 columns (separation range, 10–600 kDa), but this method does not differentiate phosphorylated or oxidized cTnI forms. Elution was performed in 0.05 mol/L phosphate buffer (pH 7.0) containing 0.15 mol/L NaCl as recommended by the manufacturer (flow rate, 0.4 mL/min; plasma sample, 0.5 mL; fraction volume, 0.5 mL). To avoid any change in cTnI forms after sampling, the fractionation procedure was performed immediately after blood collection. For molecular mass measurement of the cTnI forms, calibration was performed by measuring IgG (160 kDa), CK (85 kDa), and myoglobin (17 kDa) in each elution sample, providing only approximate masses. IgG and myoglobin were measured by immunoassays (BN II; Dade-Behring) and total CK activity was assessed at 37 °C according to IFCC recommendations with a LX-20 analyzer (Beckman Coulter).

A typical chromatogram obtained for cTnI (also measured on the DPC Immulite) in a sample obtained 120 min after PTCA (patient A, with RCA; see Table 1) is shown in Fig. 1A, with the corresponding chromatograms for IgG, CK, and myoglobin shown in Fig. 1B. Unexpectedly,
sampling fractionation revealed two major cTnI peaks corresponding to molecular masses of ~440 and 80 kDa, respectively. Although the 80-kDa mass corresponds to the cTnT-cTnI-cTnC mass, it should be pointed out that the experimental conditions used cannot separate with high resolution binary and ternary complexes. Indeed, the original aim of this work was to identify degraded cTnI forms. The macromolecular 440-kDa immunoreactive cTnI form corresponded to ~24% (15 μg/L) of the measured cTnI, and such a macromolecular cTnI form was also identified 120 min after PTCA in the two other patients tested [Fig. 1, D and E, for patients B and C, respectively (LAD)]. Thus, a macromolecular cTnI form is released into the circulation, and the form is not dependent on the location of the occluded coronary artery. To specify whether changes in cTnI forms appeared after reperfusion, we also performed FPLC fractionation 30 min after PTCA for patient B. As shown in Fig. 1D, the macromolecular cTnI form decreased from 60% to 40% of the measured cTnI between 30 and 120 min, strongly suggesting that the form gradually decreased after reperfusion. FPLC fractionation was therefore performed on a sample from patient A collected 48 h after PTCA with a cTnI concentration of 24.4 μg/L. The macromolecular cTnI form decreased from 24% to <5% (compare Fig. 1, panels A and C), and we were unable to detect such a macromolecular form 72 h after PTCA in the three patients.

As shown in Fig. 1E (patient C), we observed a third peak with a molecular mass of ~130 kDa. This peak was observed in samples from two other patients, but the peak was partially embedded in the 80-kDa peak (Fig. 1, A and D) and totally disappeared at 48 h (Fig. 1C). Unexpectedly, we observed very low concentrations (<0.5%) of degraded cTnI (molecular masses <17 kDa; fractions 61–64 and 72–76 in Fig. 1A). We have no clear explanation for the absence of observed degraded cTnI forms, and we can only speculate that this may be, for example, a
consequence of the inability of the antibodies used to recognize such degraded forms and (or) the rapid initiation of PTCA, which was performed within 6 h after onset of the chest pain.

To further characterize the complexes, we measured both cTnI and cTnT in fractions from a sample obtained 120 min after primary PTCA in another patient with occluded RCA. cTnT was measured on an Elecsys (Roche Diagnostics; URL = 0.1 µg/L, ROC curve). As shown in Fig. 1F, cTnT was present in all the fractions containing cTnI. However, we cannot conclude that cTnT is complexed with cTnI, and further characterizations are therefore required. Finally and for this patient, we tested the effect of incubation with a reducing agent [3 mmol/L dithiothreitol (DTT)] for 30 min at 25 °C on cTnI from the pooled fractions of each peak. DTT treatment induced an increase (1.7-fold) in cTnI for the 440-kDa complex (pooled fractions 34–37). FPLC fractionation of the DTT-treated 440-kDa complex showed that DTT treatment induced no change in the molecular mass of the complex (data not shown). This indicated that the reducing agent was able to expose new sites recognized by antibodies but was unable to modify the complex composition under these experimental conditions (i.e., 30 min at 25 °C). Contrary to this, DTT treatment induced a decrease in cTnI for both the 130-kDa (0.85-fold; pooled fractions 42–44) and 80-kDa (0.70-fold; pooled fractions 46–50) complexes.

In conclusion, the data presented here demonstrated macromolecular cTnI complex release in AMI patients after successful PTCA. Further investigations are required to determine the structure of the complex.

We gratefully acknowledge Jocelyne Guignery and Sylvie Marinier for helpful technical assistance and Dr. François Bureau (Laboratoire de Biochimie A, Centre Hospitalier Universitaire et Régional de Caen, France) for cTnI measurements.

References


Rapid Genotyping of Melanocortin-1 Receptor with Use of Fluorescence-labeled Oligonucleotides, Konstanze Diefenbach,1 Przemyslaw M. Mrozikiewicz,1 Britta Brien,1 Olvert Landt,2 and Ivar Roots1 (1 Institute of Clinical Pharmacology, Charité, Humboldt University of Berlin, 10117 Berlin, Germany; 2 TIB Molbiol, Syntheselabor, 10829 Berlin, Germany; * address correspondence to this author at: Institute for Clinical Pharmacology, University Hospital Charité, Schumannstrasse 20/21, 10117 Berlin, Germany; fax 49-30-450-52932, e-mail konstanze.diefenbach@charite.de)

The melanocortin-1 receptor (MC1R), localized on chromosome 16q24.3, is a G-protein-coupled receptor expressed mostly in melanocytes. Melanotropic ligands such as α-melanocyte-stimulating hormone and adrenocorticotropic hormone act via MC1R and regulate the proportion of the photo-protective melanins eumelanin and pheomelanin, which may contribute to ultraviolet (UV) radiation-induced skin damage (1) by favoring the synthesis of eumelanin. Individuals with red hair have a predominance of pheomelanin in hair and skin and/or a reduced ability to produce eumelanin, which may explain why they fail to tan and suffer from increased cutaneous UV sensitivity and why UV irradiation is more dangerous for them. Fair skin and red hair are also associated with an increased risk of cutaneous malignant melanoma (2, 3). Recently, some polymorphic variants of MC1R have been associated with red hair and found to be overrepresented in individuals with fair skin (4–7), particularly the Arg151Cys, Arg160Trp, and Asp294His variants (1, 4). These variants correlate with an increased risk of malignant melanoma (7–9). The association between MC1R variants and malignant cutaneous melanoma suggests that the MC1R gene is a susceptibility gene for this skin malignancy (10).

Heritable factors should be taken into consideration when measuring cutaneous UV sensitivity of substances with a phototoxic potential. Because MC1R may be a genetic determinant of individual skin sensitivity toward UV irradiation, we developed LightCycler assays for the detection of common MC1R polymorphisms. This rapid-cycle PCR combined with real-time fluorescence monitoring and melting point analysis is able to detect polymorphisms in ~1 h.

LightCycler methods were established for all common (>1%) and/or functional polymorphisms (11–13) (see