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Biomarker Discovery and Profiling

Unbound Free Fatty Acid Concentrations Are Increased in Cardiac Ischemia

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Abstract

Monitoring increased plasma unbound free fatty acid (FFA_u) concentrations has been proposed as a biomarker for myocardial ischemia. In the current study, 30 acute coronary syndrome (ACS) patients presenting in the emergency department, with chest pain within 12 h of onset, were clinically evaluated along with serial cardiac troponin I (cTnI) and FFA_u measurements.

Increased FFA_u were found in 28 of 30 (93%) of ACS patients, ranging from 2.0 to 430 n*M*. For the nine ACS patients with myocardial infarction (MI), FFA_u levels were increased at presentation for all (100%). In contrast, cTnI was increased in only 9 of 30 (30%) patients, mean 0.7 µg/L, and in only 2 of 9 (22%) MI patients, mean 1.3 µg/L. During the 24 h following admission, cTnI increased in all 9 MI patients. FFA_u concentrations increased in every sample in which cTnI increased.

Our findings suggest that FFA_u is increased in ischemia regardless of the presence or absence of myocardial necrosis, as reflected by increased or normal cTnI, respectively.

Key Words: Unbound free fatty acids; ischemia; myocardium; cardiac troponin.

Introduction

Myocardial ischemia (MI) generates a variety of cellular events within minutes of the interruption of blood flow that stimulate the release of plasma free fatty acids (FFA). Increases of total plasma FFA concentrations are found in patients with acute MI shortly after the event *(1,2)*. A method has been developed to measure the unbound (FFA_u) fraction

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of total plasma FFA *(2–4)*. As demonstrated earlier, levels of FFAu increase within 30 min of balloon inflation in patients undergoing percutaneous transluminal coronary angioplasty (PTCA), even in the absence of necrosis *(5)*. To evaluate the potential diagnostic utility of FFA_u, plasma FFA_u and cTnI were measured in patients presenting with chest pain presumed to be of myocardial origin.

Methods

The study population comprised 30 patients between the ages of 35 and 79 yr, 18 men and 12 women, presenting with chest pain to Hennepin County Medical Center's emergency department between April 1998 and June 1999. All patients presented with sustained chest pain of at least 10 min within the preceding 12 h and had a clinical diagnosis from the attending physician of ischemia. However no standard confirmation test for detecting ischmia was available; neither were stress tests or cardiac catheterizations. Eight of these patients had additional diagnoses that have been associated with myocardial injury (MI) (i.e., trauma 4, cocaine-2, third-degree burns-1, sepsis-1). All patients were evaluated with serial cTnI monitoring at presentation (0 h) and additional samplings during hospitalization. Nine patients had a diagnosis of an MI established by their cardiologists using ECG and cTnI values that were available to the clinicians at the time. Patients were enrolled prior to release of the ESC/ACC consensus guidelines for the redefinition of MI in September 2000 *(6)*, and thus the criteria used by the local physicians for MI diagnosis followed World Health Organization (WHO) guidelines *(7)*. The remaining 21 patients had chest discomfort consistent with angina pectoris, and 17 of these 21 had abnormal electrocardiograms (ECG)s consistent with ischemia.

Plasma (heparin) was obtained at presentation and at least two more times during hospitalization. All samples were frozen and maintained at –70°C prior to analysis. Samples were blinded for measurements of FFAu, cTnI, and patient diagnoses. cTnI for the purposes of this evaluation was measured using the Ortho Clinical Diagnostics (OCD) Vitros cTnI assay *(8)*, with an upper reference limit of 0.12 μg/L, defined at the 10% imprecision concentration *(9)*. FFA_u was determined using the ADIFAB2 fluorescent molecular probe, as described previously for the earlier ADIFAB probe *(3,4)*. The present measurements with ADIFAB2 were done at 22°C, were not affected by hemoglobin, and showed improved low end sensitivity. Duplicate measurements were done for each sample, with a coefficient of variation of 7%. The FFA_u reference limit (determined from 48 healthy subjects) was < 2.7 n*M* (mean 1.5 [SD 0.6] n*M*; range 0.6–4.5 n*M*). The institutional review board approved the protocol.

Results

Levels of FFA_u were increased at presentation in 28 of 30 (93%) patients with chest pain; range of all values were from 2.0 to 430 n*M*, mean 31 (SD 61) nM (Fig. 1). All nine patients with a diagnosis of MI had increased FFA_u at presentation. Of the two non-MI patients without an increased FFA_u at presentation, one revealed increased levels within 6 h (change from 2.5 to 4.3 n*M*); whereas the other never increased above 2.6 n*M* over 3 h. FFA_u remained increased for 6 to 64 h in the 29 patients with increased concentrations.

Using reference limit criteria for levels of cTnI measured with the OCD assay, it was determined that at presentation cTnI was increased in 7 of 21 patients (33%) classified as not having a MI (mean 0.7 [SD 1.8] μg/L) and in only two of nine MI patients (22%) (mean 1.3 [SD 3.0] μg/L). cTnI became increased in all nine MI patients and in an additional two non-MI patients (9 of 21 overall) over time.

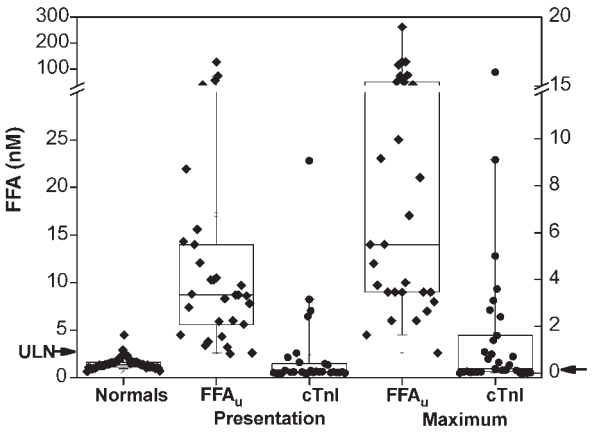

Fig. 1. FFA$_u$ and cTnI concentrations in normal subjects and chest pain patients. FFA$_u$ are represented as diamonds and cTnI as circles. Normals are shown for FFA$_u$ only. Box plots indicate median and 25th and 75th percentiles of FFA$_u$ for normals; FFA$_u$ and cTnI at presentation; maximum FFA$_u$ and cTnI over hospitalization. ULN = upper limit of normal.

FFA$_u$ were increased in every sample in which TnI was increased.

Discussion

These data suggest that plasma FFA$_u$ are increased in patients presenting with ischemic, chest pain presumed to be of cardiac origin. Previous studies of chest pain patients demonstrated that total plasma FFA$_u$ are increased in MI patients *(1,2)*. Previous studies have also shown that FFA$_u$ concentrations were increased significantly 30 min after PTCA balloon inflation and preliminary results indicate increased FFA$_u$ in patients with transmural MI and acute coronary syndromes *(5,10,11)*.

The present study suggests that FFA$_u$ is increased by ischemia regardless of the presence or absence of necrosis, as reflected by the absence or presence of increased cTnI. FFA$_u$ was increased in every instance that cTnI was increased and there was a trend towards positive correlation between peak FFA$_u$ and cTnI concentration. Additionally, in MI patients, FFA$_u$ was increased in 100% patients at presentation, whereas only 22% of these patients revealed increases in cTnI at presentation, indicative of the earlier appearance of this analyte in the circulation before traditional markers of myocyte necrosis. Some of these patients were ultimately diagnosed with additional diagnoses that can cause myocardial ischemia and injury (such as sepsis, cocaine abuse, and cardiac contusion) independent of plaque rupture. This suggests that levels of FFA$_u$ may increase early in the presence of acute myocardial injury and ischemia independent of plaque rupture. Further studies are warranted to evaluate the potential clinical utility of FFA$_u$ in the evaluation of patients who present to the emergency department with chest pain. We recognize the limitations of the lack of an objective standard for detecting ischemia and sparse clinical information available. However, the potentially important finding of this this study is the early detection of ischemia with FFA$_u$.

In summary, our data suggests that in patients presenting with ischemic, chest pain plasma FFA$_u$ monitoring may provide an early indication of cardiac ischemia in the absence or presence of necrosis based on cardiac troponin I monitoring. We demonstrated that nine of nine MI patients had an increased FFAu vs two of nine for cTnI. In chest pain patients, 93% had an increased FFA$_u$ compared to increases in 30% for cTnI.

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