The serial changes in plasma homocysteine levels and it's relationship with acute phase reactants in early postmyocardial infarction period

Erken miyokard infarktüsü sonrası dönemde plazma homosistein düzeylerindeki seri değişiklikler ve akut faz reaktanları ile ilgisi

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ABSTRACT

Objective: We aimed to study the change in the plasma homocysteine concentration in the early stage of acute myocardial infarction and its relationship with the acute phase reactants.

Methods: We included into the study 33 patients who were admitted to the hospital with acute myocardial infarction within the first three hours after the onset of symptoms. The plasma samples were obtained on admission (within 3 hours onset of symptom) and at 6, 12, 24 hours and 2, 4, 7, 30 and 90th day after admission.

Results: The serial homocysteine measurements were as following: 11.87±0.71 µmol/L, 11.89±0.62 µmol/L, 11.37±0.83 µmol/L, 10.96±0.93 µmol/L, 11.37 ±0.89 µmol/L, 11.24±0.66 µmol/L, 13.09±0.64 µmol/L, 12.85±0.71 µmol/L and 12.19±0.91 µmol/L respectively (p=0.05). Statistically significant difference was found only between the hour 24 and the day 7 (p=0.04). However, there was no statistically significant difference between the admission level and none of the other time points. No correlation was identified between acute phase reactants and lipid parameters that were measured serially at the same time periods and homocysteine levels.

Conclusion: Although homocysteine plasma values obtained during the sixth and twelfth hours of acute myocardial infarction provide reliable results as a risk markers, timing of blood sampling during the myocardial infarction does not have significant role since plasma values of homocysteine did not affect acute phase reactants. *(Anadolu Kardiyol Derg 2005; 5: 8-12)*

Key words: Acute myocardial infarction, homocysteine, acute phase reactants

ÖZET

Amaç: Bu çalışmada erken miyokard infarktüsü sonrası dönemindeki plazma homosistein düzeylerindeki seri değişimler ile bunların akut faz reaktanları ile ilişkisini araştırmayı hedefledik.

Yöntemler: Bu amaçla akut miyokard infarktüsünün semptomlarının başlamasından hemen sonraki 3 saat içinde başvuran 33 hastayı çalışmamıza aldık. Kan örneklerini gelişte (semptomların başlangıcından itibaren ilk 3 saat içinde) ve gelişten hemen sonraki 6, 12, 24'üncü saatlerde ve 2, 4, 7, 30 ve 90. günlerde topladık.

Bulgular: Seri homosistein ölçümlerinde homosistein düzeyleri sırasıyla 11.87±0.71 µmol/L, 11.89±0.62 µmol/L, 11.37±0.83 µmol/L, 10.96±0.93 µmol/L, 11.37±0.89 µmol/L, 11.24±0.66 µmol/L, 13.09±0.64 µmol/L, 12.85±0.71 µmol/L ve 12.19±0.91 µmol/L olarak saptadık (p=0.05). İstatiksel olarak anlamlı farklılık yalnızca 24. saat ile 7. gün değerleri arasında görüldü (p=0.04). Bununla birlikte geliş düzeyi ile hiçbir zaman kesiti arasında istatistiksel olarak anlamlı ilişki bulunmadı.

Sonuç: Plazma homosistein düzeyleri akut faz reaktanlarını etkilememekte ve risk belirleyicisi olarak homosisteinin akut miyokard infarktüsünün ilk 6. ve 12. saatlerinde ölçülen plazma değerleri güvenilir sonuçları vermekle birlikte kanın alınma saati, klinik açıdan önemli değişkenlik getirmemektedir. *(Anadolu Kardiyol Derg 2005; 5: 8-12)*

Anahtar kelimeler: Akut miyokard infarktüsü, homosistein, akut faz reaktanları

Introduction

It is well known that serum lipid parameters have an important role in the risk stratification for coronary artery disease (1). The meta-analysis of the large population studies carried out recently revealed that homocysteine is also a serious risk factor (2). Raised plasma homocysteine concentrations facilitate thrombosis during acute coronary events (3). Furthermore, patients with increased homocysteine are at significantly greater risk of long-term mortality (5,6). These results suggest that ho-

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mocysteine lowering treatment may need to be considered during risk modification after acute myocardial infarction (MI). Since plasma homocysteine levels may be important elements that have to be taken into account in the arrangement of a treatment following the MI, such factors should be measured reliably. However, biochemical assays performed during an acute coronary event have been known to give false results and to the acute phase reactions. For example, reliable estimates of total cholesterol can be obtained within 12-24 hours of the onset of symptoms, but concentrations fall by 20-40% on days 4-5 and subsequent estimates may not be reliable again until two months after acute MI. Measurement of the plasma homocysteine level in all patients after hospital admission for acute MI may become standard practice for risk stratification. The serial changes in the plasma homocysteine concentration following acute coronary syndromes were reported rarely. Several studies reported no significant changes during acute events (7,8). Furthermore, in these studies, the initial blood samples were taken at least 24-48 hours from the onset of symptoms. There is no study that has been conducted in the earlier stages of acute MI and in more frequent time points. In this study we investigated the serial plasma homocysteine level changes during the acute phase and the long-term in patients with acute myocardial infarction. Our aim was to estimate the timing of blood sampling which may reliably reflect the pre-infarction plasma homocysteine levels and the associated risk. The correlation between the changes in the plasma homocysteine levels and the acute phase reactants were also investigated.

Materials and Methods

The patients included in to the study were those with acute MI who had been referred to the intensive care unit of our teaching hospital within the first 3 hours of the chest pain and had ST-segment elevation on the electrocardiogram. All 33 patients were admitted only during early morning hours (between 06:00 and 07:00 am) and were in a fasting state. Patients who had the habit of using vitamins before this acute event, patients who were alcohol addicts and patients with renal, hepatic and thyroid diseases were excluded from the study. The informed consent was obtained from all the patients, with the approval of local ethics committee. Acute myocardial infarction diagnosis was made according to the criteria of World Health Organization (WHO); typical chest pain, creatine kinase-MB elevation two times above the normal limits and ≥1 mm ST-segment elevation in the two neighboring extremity leads and ≥2 mm ST-segment elevation in the two neighboring precordial leads (9). All the details related to clinical features were recorded for all patients. Clinical details included the evaluation of risk factors for the coronary artery disease. The issue of smoking was classified as current smokers and non-smokers. The current smokers included those who stopped smoking less than 1 month before enrollment into the study. The known history of diabetes mellitus or hypertension, and details of treatment received before admission were recorded. Patients who had a new acute coronary event (unstable angina pectoris or MI) in the first 3 months of the follow-up after discharge were excluded from the study.

The venous blood samples were taken from patients at referral by a trained medical intensive care nurse from the antecubital vein before starting thrombolytic and anticoagulant therapy. The blood samples were transferred into tubes containing EDTA for the analysis of homocysteine, hemogram and erythrocyte sedimentation rate and into tubes which did not contain anticoagulants for other analysis. Within 15 minutes after the sample was taken, platelet-poor plasma/serum was obtained through centrifugation at room temperature and was immediately transferred to a freezer at –40∞ C. Serial blood samples were obtained from all patients at 3, 6, 12, 24, 48 (day 2), 96 (day 4), 168 (day 7) hours on admission and again at day 30 and 3 months after the acute MI. The samples were collected between 09:00-10:00 Am when the patients were fasting and were stored as defined above. The blind analysis of all samples was carried out at one time by the laboratory staff who had no information on the patients and the design of the study.

Homocysteine measurement: Plasma total homocysteine level that includes the sum of protein bound and free homocysteine was measured by high performance liquid chromatography method, which was identified by fluorescence (10,11). Intra- and inter-coefficient variations were 5% and below. Plasma homocysteine was measured as µmol/L unit.

Measurement of acute phase reactants: The analysis of C-reactive protein (CRP), complement 3 (C3), complement 4 (C4), alpha-1 antitrypsin, alpha-2 macroglobulin were carried out by using Behring Nepholometer 100 Analyzer and Dade Behring immunophelometric kits (Dade Behring Germany, Marburg, Germany) according to the protocol recommended by the company.

The measurement of lipid parameters: The measurements of total cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), high-density lipoprotein (HDL) and triglyceride were conducted by the standard enzymatic methods. The analysis of apolipoprotein A (Apo A), apolipoprotein B (Apo B) and lipoprotein (a) were made by Behring Nepholometer 100 Analyzer and Dade Behring immunophelometric kits (Dade Behring Germany, Marburg, Germany). The samples collected for measuring hematocrit, hemoglobin and leukocyte count were studied in Cell-Dyn 3500 R, Abbot.

Statistical Analysis: The values were given as standard error of mean (SEM). Variance analysis (ANOVA) was used to compare the serial measurements. Dunnett test was used as a post hoc analysis for multi-comparison of significant results. The changes in plasma homocysteine were expressed as a change from the referral value in percentages. The correlation was tested by Pearson correlation test. In all the results obtained, $p<0.05$ was considered to be statistically significant.

Results

The demographic characteristics of all patients (n=33) are given in the Table 1. There was a classical rise, which a peak in CRP and alpha-1 antitrypsin levels on day 2, and returned to normal on the month 3 ($p<0.0001$) (Table 2). The significant rise in CRP, when compared to the value at referral, began after the first 12 hours (for hours 24, 48, 96, 168 $p<0.0001$ vs. hour 3 and for day 30, $p=0.01$ vs. hour 3). A similar curve, which reached the peak on day 3 and returned to normal in 3 months, was observed in the erythrocyte sedimentation rate (Table 3). However, this fluctuation was not statistically significant. Alpha-1 antitrypsin started to rise after the first 12 hours like CRP and reached a peak level at 48 ($p=0.002$) and 96 hours ($p=0.008$). The rise in the plasma concentration in C3 and C4 began in the first 24 hours

and reached a peak at the end of 1st week (168 hours) (for C3-p<0.0001 vs. 3 hr, 6 hr, 12 hr and p=0.005 vs. 24 hr) and returned to normal on the month 3. Figure 1 shows the change in homocysteine concentration following myocardial infarction. In comparison with admission the homocysteine level (in the first 3 hours after the onset of symptoms), homocysteine plasma level started to decrease at hour 12 (11.37 ±0.83 µmol/L) and at hour 24 (10.96±0.93 µmol/L). On the contrast these values increased after first day by 0.2% that in the seventh day, as compared with admission values. The last level of homocysteine at the end of a period of three months was higher than referral by 2.6% (12.19±0.91 µmol/L). There was a slight statistical difference when all groups were examined together (p=0.05) (Table 1). The post-hoc analysis conducted using Dunnett test revealed only a slight but statistically significant difference between hour 24 and day 7 (p=0.04). Statistically, there was not significant difference between the first level of homocysteine and any value obtained at other time points. Correlation analysis revealed a significant relation between homocysteine concentration on admission and levels at hour 6 (r=0.48, p=0.0001), hour 12 (r=0.35, p= 0.0001), hour 24 (r= 0.49, p= 0.0001), hour 48 (r=-0.29, p=0.0001) and hour 168 (r=0.30, p= 0.0001). Plasma alpha-2 macroglobulin level decreased until hour 24 as homocysteine and then increased and reached the starting level on day 30, but these changes were in significant. Additionally, no correlation was established between homocysteine measured serially and acute phase reactants. When lipid parameters in acute period were examined; a regular decrease was observed starting from hour 12 when compared to the first value in total cholesterol and LDL cholesterol, and the largest decrease took place at hour 48, during serial measurements (Table 4). Such values increased on the day 30 and reached a level close to the first one on month 3. Triglyceride and VLDL levels decreased slightly at hours 6 and 12, and then increased gradually in the following serial measurements and were not significantly different from the first level. A decrease was observed in plasma Apo A and Apo B concentrations at 24 hours, and there was not any difference between measurements at other times.

Table 1. Clinical and risk profile characteristics of the study group

Number of patients	33
Sex (men/women), n	27/6
Mean age, years	56.6±1.4
Ejection fraction, %	50.6 ±1.0
Previous angina, n(%)	7 (21)
Smoking	
Current, n(%)	14(42)
Non-smoker, n(%)	10(30)
Thrombolytic therapy (STK/t-PA),n	9/18
Diabetes mellitus, n(%)	3(9)
Hypertension, n(%)	12(36)
Localization of MI(inferior/anterior),n	20/13

CK; creatine kinase, MI; myocardial infarction, STK; streptokinase, t-PA: tissue plasminogen activator

Discussion

Many systemic changes occur in acute illness period and these changes are called as 'acute phase response' in general and are accompanied by an increased synthesis of hepatic proteins such as serum CRP, alpha-1 antitrypsin (12). As we have indicated in our study, an increase is observed in levels of many acute phase proteins in acute MI. Senaratne et al. established that the level of homocysteine within 48 to 72 hours of acute MI was higher than the levels on week 6 and suggested that this might be related to the increase in acute phase reactants. In our study, this issue has been specifically examined and no relation has been found between acute phase reactants and homocysteine. Additionally, it has been reported that acute phase proteins may be combined with homocysteine and that it can change free and protein-dependent homocysteine level due to the dependence of homocysteine on albumin. However, a study in which total and free homocysteine levels have been measured

Table 2. Serial measurements results of acute phase reactants in the study group

	3rd hour	6th hour	12th hour	24th hour	48th hour	96th hour	7th day hour	30th day	90th day	p
HCY, mmol/L	11.87±0.71	11.89±0.62	11.37±0.83	10.96±0.93	11.37±0.89	11.24±0.66	13.09±0.64	12.85±0.71	12.19±0.91	0.05
CRP, mg/L	5.4±2	7.7±2	15.6±2	42.9±5	54.9±5	50.5±6	40.2±6	10.1±3	6.3±1	<0.0001
C3, mg/dl	104.0±3	103.7±3	105.0±4	114.8±5	122.2±5	118.7±4	135.3±6	115.2±6	102.3±4	<0.0001
C4, mg/dl	22.8±1	23.0±1	22.8±1	25.4±1	27.8±1	28.6±1	33.0±2	25.1±2	21±1	<0.0001
AAT, mg/dl	145.7±7	148.8±5	160.5±9	177.3±9	203.7±13	196.9±11	190.4±12	171±10	147±8	<0.0001
AMG, mg/dl	186.1±14	159.1±12	148.4±9	141.4±8	145.0±10	156.6±12	169.8±13	174±11	181±13	0.09

AAT: alpha-1 antitrypsin, AMG: alpha-2 macroglobulin, C: complement protein, CRP: C-reactive protein, HCY: Homocysteine

Table 3. Serial measurements results of cardiac enzyme and blood parameters in the study group

	3rd hour	6th hour	12th hour	24th hour	48th hour	96th hour	7th day	30th day	90th day	p
CK, IU/L	652.3±135	1509.3±136	2077.8±195	1455.4±156	657.3±121	283.1±57	153.0±38	110±12	120±13	0.0001
LDH, IU/L	287.6±25	445.6±39	614.7±47	635.0±55	533.8±27	387.5±18	251.1±5	90±7	78±5	0.0001
ESR, mm/h	8.5±2	10.7±2	10.6±2	13.5±5	13.0±5	19.2±6	19.7±6	7±1	5±1	0.11
L, Kum/L	11.9±0.8	11.8±0.6	11.2±0.5	10.4±0.5	9.4±0.5	9.1±0.5	8.4±0.4	7.6±0.5	7.8±0.3	0.0001
HTC, %	42.7±1	40.9±1	41.7±0.9	39.7±1	40.6±1	40.9±0.8	41.1±0.8	39±1	41±0.9	0.44

CK; creatine kinase, ESR; erythrocyte sedimentation rate, L; leukocyte, LDH; lactate dehydrogenase, HTC; hematocrit,

separately established the same amount of change in both forms during acute period of MI. (14).

Meta-analysis of 27 case-controlled retrospective studies revealed that high levels of homocysteine increased the risk of fatal or non-fatal atherosclerotic vascular disease 1.7 times in coronary arteries, 2.5 times in cerebral circulation and 6.8 times in peripheral circulation (2). It was reported that there was a linear relation between homocysteine and vascular risk and an increase in homocysteine by 5 µmol/L increased the vascular risk by 3 times (2). An increase in homocysteine by 5 µmol/L corresponds to the increase in plasma cholesterol by 19 mg/dL. Additionally, it has been concluded that 10% of risk of coronary heart disease in general population may be attributed to homocysteine. It has also been indicated reduction of total homocysteine by 4 µmol/L, may decrease cardiovascular mortality, and this can be achieved daily folate consumption in dose of 200 mg.

In a recent study, homocysteine levels of patients with Q wave and non-Q wave MI, and unstable angina pectoris have been observed at the time of the hospital admission, and these patients have been followed-up for 2 years (15). Of 579 patients, 65 died during this period. Homocysteine levels of dead patients were higher than those of alive patients on admission and relative risk for mortality due to all reasons in the patients with high levels of homocysteine was 2.4. On the basis of these results, it has been suggested that plasma homocysteine level of the patients at the time of the admission is an independent predictor of long-term prognosis. In a acute coronary syndromes risk of cardiac event has been found to be 2.6 times higher in patients with a homocysteine level of >12.2 µmol/L (3). Another study revealed a significant relation between homocysteine and thrombin and homocysteine and Factor VIIa in patients with acute coronary syndromes (2). This my be an indication of prothrombotic effect of homocysteine.

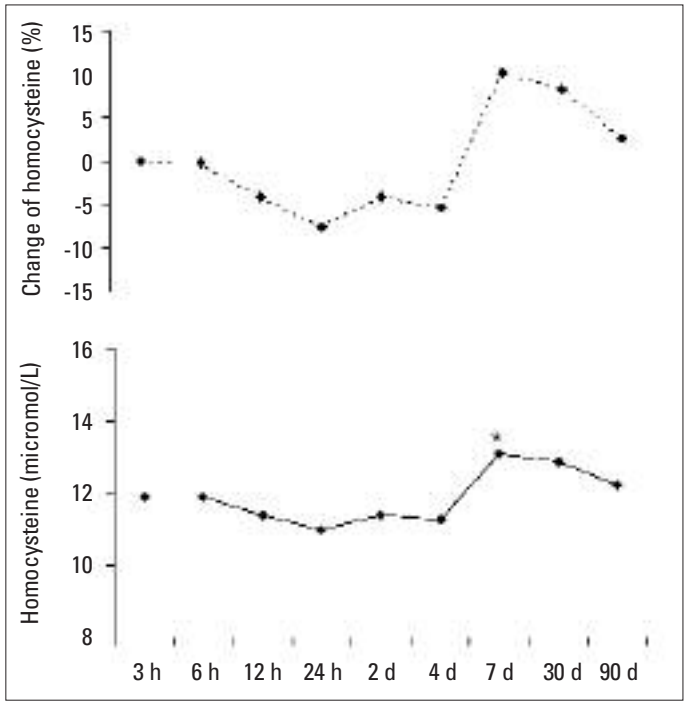

Figure 1. Plasma homocysteine variations following acute myocardial infarction. Interrupted line represents the percentage change of homocysteine level according to the 3rd hour measurement. h; hour, d; day *Dunnett post hoc analysis, hour 24 vs. day 7, p<0.05.

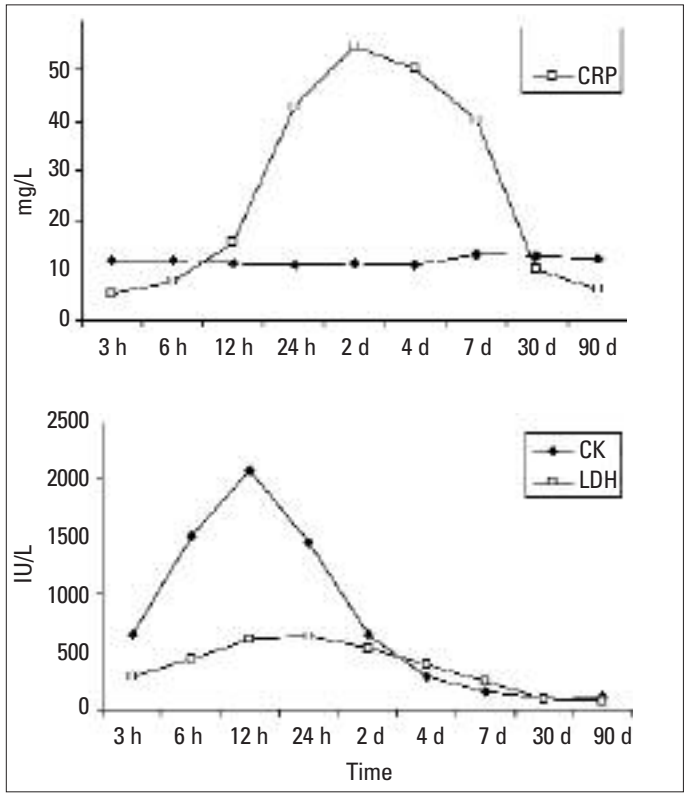

Figure 2. C-reactive protein (CRP), creatine kinase (CK) and lactic dehydrogenase (LDH) variations following acute myocardial infarction, h: hour, d: day

Table 4. Serial measurements results of lipid parameters in the study group

	3rd hours	6th hours	12th hours	24th hours	48th hours	96th hours	7th day	30th day	90th day	p
TC, mg/dL	197.6±6	196.0±6	191.3±6	190.0±5	186.3±8	189.6±4	188.1±6	191±7	182±5	0.86
LDL, mg/dL	125.2±5	125.1±6	121.5±5	113.9±4	109.1±7	114.7±4	108.2±8	111.9±6	115.2±8	0.26
HDL, mg/dL	41.9±1	43.1±1	44.7±1	45.5±1	43.7±1	41.1±1	39.3±1	42±2	40±1	0.22
VLDL, mg/dL	32.0±3	29.2±2	28.7±2	30.8±3	35.0±3	35.7±2	35.7±2	33±3	31±2	0.40
TG, mg/dL	150.8±13	145.6±14	145.8±13	159.1±15	175.7±15	169.7±11	184.3±12	172±11	155±10	0.35
Lp(a), mg/dL	28.1±6	27.2±5	26.9±5	32.0±7	28.2±8	33.6±7	28.8±6	30±7	27±4	0.99
Apo-A, mg/dL	131.9±4	131.6±3	128.0±5	131.1±3	137.0±4	121.6±4	120.9±5	125±3	128±5	0.15
Apo-B, mg/dL	116.8±5	116.2±5	117.1±6	110.9±4	115.4±4	116.4±4	118.5±5	114.5±5	116.1±5	0.97

Apo; apolipoprotein, HDL; high density lipoprotein, LDL; low density lipoprotein, Lp(a); lipoprotein (a), TC; total cholesterol, TG; triglyceride, VLDL; very low density lipoprotein

In patients with stroke, it was reported that homocysteine concentration was higher by 4% at 6 weeks, by 19% at 6 months and by 27% at 18 months, when compared with initial values obtained between 24 and 48 hours after onset of an acute vascular event (7). These results are controversial with a 2-3% of difference between the levels of homocysteine at healing periods and values on admission obtained by Al-Obadi et al. (8). Maximum change in homocysteine concentration in their study was 7% and appeared between day 2 and day 28 after the onset of the chest pain. In our study, the difference between the values at the time of admission and healing periods was 2-3% and such values were attained within three months. Al-Obadi et al., on the other hand reported the results of six months (8). Additionally, the greatest difference was 19.7% and was observed between 24 and 144 hours (day 7) and the difference between plasma homocysteine concentration on admission and day 7 was 10%. Blood samples were obtained on day 2 of acute event in many of the previous studies conducted up to now. Our study, for the first time, homocysteine levels were measured at 3, 6, 12 and 24 hours. In homocysteine levels at 3 and 6 hours were almost the same, and the first decrease was observed at 12 hours and became most apparent at 24 hours; then, it increased again and reached the highest level on the day 7. Egerton et al (14) were able to observe the increase only on the day 7 since they have did not obtain the homocysteine levels at 24 hours. Therefore, they reached an incomplete conclusion reporting that homocysteine levels increased following the acute event. Although a statistically significant difference was found between serial measurement of homocysteine levels in this study, there was not any statistically significant difference between the values obtained on admission and during healing periods in our study.

Conclusion

Based on the results of our study, we can argue that plasma homocysteine levels in acute myocardial infarction are not related to the changes in blood concentrations of acute phase reactants. Homocysteine levels observed within the first 6 hours are the most reliable results to make the risk stratification of the patients. Homocysteine concentrations in blood samples taken from the patients, who were not admitted to the hospital within 6 hours, may be found to be lower or higher as it is the case on the day 7. However, these changes are not so significant to produce misleading results in making the risk stratification. Plasma homocysteine concentration on the month 3, on the other hand, is rather close to the plasma concentration taken within the first 6 hours of acute myocardial infarction.

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