Evaluation of ischemia-reperfusion damage during coronary angioplasty. Electrocardiographic assessment and biochemical modifications in blood from the coronary sinus

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Background. **Percutaneous transluminal coronary angioplasty (PTCA) constitutes a clinical model of reperfusion following a short period of ischemia connected to balloon inflation during the procedure. During the procedure some ischemic damage and oxidative injury related to free radical attack might occur. In the present study we investigated the extent of ischemic damage and some biochemical indexes of reperfusion damage in patients undergoing PTCA.**

Methods. **Twenty-five patients who underwent PTCA because of angiographically detected occlusion of the coronary artery were enrolled. Balloon inflation lasted from 30 to 60 s. ECG changes were monitored throughout the procedure and blood samples were taken from the coronary artery and coronary sinus before balloon inflation, and again from coronary sinus at the peak of ischemia, 2 and 10 min after reperfusion.**

Results. **During PTCA procedure angina pectoris appeared in 62.7% of patients, whereas ST-segment elevation was present in 87% of patients, regressing completely after balloon deflation. Plasma malonyldialdehyde, an index of lipid peroxidation, did not change; coenzyme Q_{10} (in its oxidized and reduced forms), vitamin E and β-carotene were also unchanged. Total antioxidant capacity and uric acid decreased upon reperfusion.**

Conclusions. **Myocardial ischemia occurring during balloon inflation is brief and regresses completely after balloon deflation. Reperfusion following a short period of acute ischemia such as in PTCA does not constitute an oxidative event detectable through a common marker of lipid peroxidation nor does it alter the concentration of lipophilic antioxidants. It only lowers hydrosoluble antioxidants therefore representing a mild oxidative insult.**

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Introduction

Ischemia-reperfusion damage presents important implications with relation to multiple pathologies and these issues are arousing increasing interest in preventive medicine[1]. The diffusion of current techniques to restore blood flow to the ischemic heart such as percutaneous transluminal coronary angioplasty (PTCA) have focused the attention on the consequences of reperfusion.

Ischemia followed by reperfusion constitutes a series of events in which the production of oxygen free radicals might overwhelm the capacity of the antioxidant systems[2,3].

The pathogenetic mechanism of myocardial ischemic damage is still not well known. Recent studies have demonstrated that damage can be caused as a result of reactive oxygen species generation or induced by intracellular calcium overload[4]. There is general agreement about the main components of the phenomenon which include: a) time of balloon inflation; b) the site of obstruction; c) the presence or absence of the collateral circulation; d) concomitant pharmacological therapy[5,6]. Regarding this point calcium antagonists are mainly known for their cytoprotective action; β-blockers can also reduce the entity of ischemia during inflation, but only if administered as pre-treatment.

Ischemic damage is usually assessed by changes in the ECG. The most frequent is a typical ST-segment change during PTCA, resulting from transmural ischemia of the district tributary of the artery where the balloon is inflated. Other ECG changes are sinus bradycardia, linked to ischemia at the sinus node level, and extrasystoles (mainly supraventricular).

The ensuing reperfusion damage would derive from the sudden reoxygenation of the ischemic area: in such a condition, production of reactive oxygen species would occur.

Studies on animal and human models[4] show that oxidative stress in this situation is correlated with the length of the ischemic period, suggesting that the latter is a critical event in determining the extent of oxidative injury. Of course the detection of free radical species during ischemia-reperfusion would help to clarify the mechanisms involved in the oxidative injury; changes in antioxidant concentration can also give some indications about the extent of oxidative stress.

In the present study we monitored a marker of lipid peroxidation, i.e. malonyldialdehyde (MDA) together with some liposoluble and hydrosoluble antioxidants and total antioxidant capacity in the blood of coronary sinus throughout the procedure of routinely performed PTCA. Among the liposoluble antioxidants ubiquinol, the reduced form of coenzyme Q_{10}, is a powerful antioxidant mainly carried by LDL; in fact it is the first antioxidant to intervene when LDL are subjected to oxidative stress[7].

We assayed three more liposoluble antioxidants, i.e., β-carotene and α-tocopherol, and uric acid, a very important hydrosoluble antioxidant for its high concentration in the blood.

Methods

Twenty-five patients (23 males and 2 females) with angiographically detected occlusion of the coronary artery were enrolled in this study. PTCA had been decided independent of this study, which was approved by the ethical committee of the hospital; all subjects gave informed consent. Most of them (Table I) had a history of myocardial infarction.

Patients were under standard medical therapy (Table II).

Table I. Clinical characteristics of the study patients.

Ex-smoker males	23
Post-menopausal females	2
Age (years)	60 – 10.5
Myocardial infarction	15/25
Dyslipidemia	13/25
Hypertension	8/25

Table II. Standard medical therapy.

Day before	During the procedure	After the procedure
Aspirin 325 mg Tiklid 250 mg × 2	Heparin 10 000 IU Nitroglycerin 0.2 mg	Aspirin 325 mg Tiklid 250 mg × 2 Tildiem × 3

Balloon inflation lasted from 30 to 60 s. Less than 10% of stenoses affected the right coronary artery; on this basis we did not attempt to statistically analyze the results according to the site of obstruction.

To assess PTCA-induced myocardial ischemia, all patients were monitored with continuous 12-lead ECG before, during and after PTCA procedure.

The resulting ECG changes were classified as belonging to anterior, inferior and non-specific ischemia. Anterior wall ischemia was defined as ST-segment elevation in 2 contiguous precordial V leads. Inferior wall ischemia was defined as ST-segment elevation in > 1 of the 3 limb leads (II, II or aVF). All the other ST-segment deviation patterns were classified as non-specific ischemia[8].

Coronary artery blood was collected before balloon inflation; venous blood was collected from the coronary sinus before balloon inflation (BV1), at the peak of ischemia (BV2), 2 and 10 min after reperfusion (BV3 and BV4, respectively). Sampling of arterial blood was performed only once, before balloon inflation. Although this might constitute a limitation, it is rather unlikely that myocardial events occurring during the short time in which the measurements were conducted are able to induce biochemical changes in the general circulation and therefore in the coronary artery.

Plasma clinical-chemical parameters. Total cholesterol, LDL and HDL cholesterol, triglycerides and uric acid were assayed in plasma samples (heparin was used as an anticoagulant) by a CX-Synchron apparatus (Beckman, Brea, CA, USA) using enzymatic kits.

Plasma liposoluble antioxidants. Ubiquinol, ubiquinone, α-tocopherol, β-carotene were assayed by HPLC equipped with electrochemical detector (EC). Aliquots of 50 µl of plasma were precipitated with 250 µl of isopropanol HPLC grade, vortexed for 1 min and centrifuged for 3 min at 1000 rpm. After centrifugation, 100 µl of the supernatant was injected into HPLC-EC system set up in the following conditions: C8 analytical column (250 × 46 nm, 5 µm particle size, Supelco-Sigma Aldrich, Milan, Italy) coupled with two C8-AZB precolumns (50 × 46 nm, 5 µm, Supelco-Sigma Aldrich, Milan, Italy). The mobile phase was prepared by dissolving LiCLO4 (10 mM) in methanol: 2-propanol: ethanol 72:8:20 v/v, flow rate 1.2 ml/min. The detection of the peak was carried out by a Coulochem detector (ESA, 5100A model) fitted with a model 5021 conditioning cell and a model 5010 analytical cell. The three elec-

trodes in series were respectively set at -0.6, -0.15, and +0.6 V. The peak quantification was performed using a pure standard solution of ubiquinone at known concentrations calculated spectrophotometrically using the extinction coefficient of 14 $M^{-1}L^{-1}$ at λ = 275 nm.

Malonyldialdehyde plasma levels. Plasma MDA was assayed using an LPO-586 test kit (Wak-Chemie Medical, Bad Soden, Germany). The LPO-586 assay is based on the reaction of a chromogenic reagent with MDA at 45 C. One molecule of either aldehyde reacts with two molecules of chromogen to yield a stable chromophore with maximal absorbency at 586 nm.

Total antioxidant capacity. The antioxidant capacity of plasma was tested using the method described by Miller et al.[9], based on the inhibition of the absorbency of radical cation of 2,2 -azinobis 3-ethylbenzothiazoline 6-sulfonate ($ABTS^+$) by antioxidants. This radical cation is generated *in vitro* by the interaction of ABTS and ferrylmyoglobin radical species; antioxidants present in plasma suppress the absorbency of $ABTS^+$ to an extent and on a time scale dependent on the antioxidant activity of sample under investigation.

The assay was standardized using a vitamin E analogue (trolox) and the total antioxidant capacity was expressed as mM trolox.

Results are expressed as average – SD. Statistical evaluation of obtained data was performed according to ANOVA repeated measure test.

Results

During PTCA procedure angina pectoris appeared in 62.7% of patients. It began after a latent period of almost 10-20 s, lasted throughout ischemia and vanished after balloon deflation. ECG monitoring showed ST-segment elevation in 83% of patients. The amplitude of ST-segment elevation was similar to that observed in clinical practice. Ischemia involved the anterior wall in 39% of cases, the inferior wall in 18% of cases, and was nonspecific in 26% of cases. Other ECG findings were supraventricular extrasystoles (12%) and sinus bradycardia (4%).

The concentration of lipophilic antioxidants, as well as the ubiquinol/ubiquinone ratio, remained unchanged throughout the procedure, as shown by the values found in BV1, BV2, BV3 and BV4. Furthermore, MDA levels did not increase in any of the different steps of PTCA procedure (Table III), suggesting that lipid peroxidation does not occur during reperfusion following short ischemia. The only parameter which showed a decrease, statistically significant in BV3, was the total antioxidant capacity value, which was mainly related to changes in hydrosoluble antioxidant concentrations. In fact uric acid showed a similar trend to total antioxidant capacity, decreasing in BV3, although without statistical significance (Fig. 1).

Discussion

The results obtained in our study group confirm that myocardial ischemia which occurs during balloon inflation is very short, and regresses completely after balloon deflation. The 12-lead ECG is usually considered

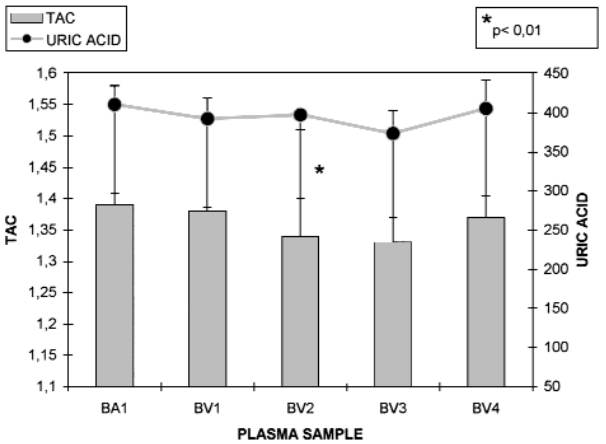

Figure 1. *Total antioxidant capacity (TAC) and uric acid levels in plasma from the coronary artery and coronary sinus at different times during coronary angioplasty. BA1 = blood sample from the coronary artery before balloon inflation; BV1 = blood sample from the coronary sinus before balloon inflation; BV2 = blood sample from the coronary sinus at peak ischemia; BV3 = blood sample from the coronary sinus 2 min after reperfusion; BV4 = blood sample from the coronary sinus 10 min after reperfusion.*

Table III. Antioxidants and malonyldialdehyde (MDA) levels in plasma.

Antioxidants	BA1	BV1	BV2	BV3	BV4
Coenzyme Q_{10} (μM)	0.85 – 0.21	0.85 – 0.20	0.83 – 0.20	0.83 – 0.20	0.84 – 0.21
Ubiquinol (%)	88 – 5	89 – 5	87 – 5	87 – 6	88 – 5
α-tocopherol (μM)	33.0 – 7.0	32.0 – 6.6	34.0 – 8.1	31.0 – 5.5	34.0 – 7.8
β-carotene (μM)	0.24 – 0.13	0.23 – 0.08	0.20 – 0.07	0.24 – 0.13	0.25 – 0.15
MDA (μM)	0.50 – 0.13	0.49 – 0.13	0.46 – 0.11	0.49 – 0.13	0.54 – 0.19

BA1 = blood sample from the coronary artery before balloon inflation; BV1 = blood sample from the coronary sinus before balloon inflation; BV2 = blood sample from the coronary sinus at peak ischemia; BV3 = blood sample from the coronary sinus 2 min after reperfusion; BV4 = blood sample from the coronary sinus 10 min after reperfusion.

a more sensitive index of ischemia than the appearance of angina[8], which was confirmed by our results. It is known that there is no correlation between the entity of angina and the amplitude of ST-segment deviation[10]. The overall benefits of PTCA in our group of patients were unquestionable. In 94% of them the stenosis area was reduced to < 30% of the cross section of the artery and to < 10% in 61% of patients.

On the biochemical side it is quite evident from our results that none of the lipophilic antioxidants changed in concentration during PTCA. Not even the ubiquinol/ubiquinone ratio, which is a sensitive marker of free radical attack, was affected by ischemia-reperfusion related to PTCA, in our conditions.

A study conducted in 13 patients undergoing PTCA reported that lipid hydroperoxide levels in plasma and LDL remained unchanged throughout the period following PTCA[11].

Previous observations on blood samples taken from the great cardiac vein before and immediately after one to five serial balloon inflations indicated a raised lipid peroxide concentration after balloon angioplasty in 59% of inflations lasting 60 s, even though there was no correlation between lipid peroxides and the degree of preceding myocardial ischemia as assessed by either ST-segment shift or lactate production[12].

Even though MDA determination represents a biochemical index which is not highly specific, if the environmental parameters are kept constant, the MDA content of a system is believed to be indicative of the extent of formation/decomposition of lipid oxidation products which are MDA precursors[13]. Usually correlative data of other indices of lipid peroxidation strengthen the value of this test.

In our work samples from the coronary sinus did not show any significant variations of MDA, nor were the lipophilic antioxidants affected. Previous data[14,15] showed some increased release of MDA during PTCA; in fact there was a direct relationship between the ischemic burden and the myocardial release of thiobarbituric-acid-reactive-substances in 8 patients undergoing angioplasty[14]. Data obtained by De Scheerder et al.[15] showed that four consecutive transluminal dilations of 90 s each with deflation intervals of 3 min were able to induce a significant increase in great cardiac vein-arterial concentrations of hypoxanthine, MDA and uric acid. It is interesting to notice that whereas MDA and hypoxanthine release is progressive and starts right after the first inflation, uric acid vein-arterial difference slightly decreases after the first two inflations and then sharply rises. In our patients the ischemic period was always shorter compared with patients described in the latter papers; in fact our balloon inflation lasted on average 45 s. In this condition we had a significant decrease of uric acid in blood from the coronary sinus shortly after reperfusion. This is in agreement with the data of De Scheerder at al.[15] and our interpretation is that after a mild oxidative stress uric acid is among the first antioxidants to counteract the oxidative insult and therefore we can see a decrease in concentration. With repetitive occlusions the uric acid vein-arterial difference increases, possibly due to enhanced hypoxanthine conversion by endothelial xanthine oxidase, overwhelming a certain degree of uric acid consumption as antioxidant.

Urinary levels of F2 isoprostanes are a family of stable end products of lipid peroxidation and they probably represent a non-invasive index of free radical generation in syndromes of cardiac reperfusion. In fact not only after PTCA for acute myocardial infarction, but also after elective PTCA there was a significant, even though slight, increase of these products[16]. They could have a cardiac origin; therefore their assay could be more sensitive than MDA or lipophilic antioxidants. They might also represent a product arising from myocardial tissue, and possibly from other sites, during the 6-hour period following elective PTCA.

Direct evidence of free radical generation, by electronic paramagnetic resonance measurement of a spin-trap adduct, was presented even in man[17]; in fact the *ex-vivo* assay of the adduct was performed in blood taken from the right atrium during angioplasty reperfusion for acute myocardial infarction.

Our assays were conducted in blood from the coronary sinus. MDA does not likely represent the most sensitive parameter of lipid peroxidation; nonetheless, these data, together with the unchanged levels of lipophilic antioxidants, might suggest that the degree of free radical production during reperfusion following such a short ischemic period, as in our procedure, is not sufficient to attack plasma lipoproteins.

Moreover, in the present study, total antioxidant capacity decreased significantly at the onset of reperfusion and this was in connection with a lowering of uric acid. It is likely that ascorbic acid, which we did not test in our study, also decreased during PTCA. Therefore routinely performed PTCA procedure seems to involve a free radical production capable of affecting water-soluble antioxidants, which constitute the first line of intervention against radical insult. In fact studies conducted in isolated rat hearts have shown that tissue hydrophilic antioxidants are easily oxidized, when lipophilic antioxidants are still unaffected, and may therefore constitute the first line of antioxidant defenses during reperfusion[18].

In conclusion, our study demonstrated that in our patients, in whom a single inflation was enough to recanalize the obstructed artery, the duration of ischemia was not long enough to result, upon reperfusion, in lipid peroxidation as detectable by plasma MDA. Lipophilic antioxidants were also unchanged, so we can reasonably hypothesize that the limited oxidative stress which occurred in our patients was well counteracted by hydrosoluble antioxidants, without a detectable insult on the lipid component of plasma. If the procedure involves a longer lasting ischemia, or in PTCA after myocardial infarction, biochemical markers of reperfusion damage become more evident.

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