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Pregnancy associated plasma protein-A (PAPP-A) is not a marker of the vulnerable atherosclerotic plaque

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ABSTRACT

Objective: To investigate if pregnancy associated plasma protein-A (PAPP-A) was present in the vulnerable plaque, and if not, to find alternative hypothesis for the release of PAPP-A.

Design and methods: Vulnerable plaques and control tissues were examined by immunohistochemistry. Volunteers and patients with non-atherosclerotic disease were examined for release of PAPP-A during ischemia and medical treatment. Non-atherosclerotic tissue samples were examined after incubation with heparins.

Results: We were not able to detect PAPP-A in vulnerable plaques. Patients and volunteers experiencing ischemic events without atherosclerotic lesions only had elevated PAPP-A when treated with heparin. When tissue from normal artery wall was incubated with heparin, PAPP-A was eluted. This was not the case for non-arterial tissue samples.

Conclusion: Elevation of PAPP-A in patients with acute coronary syndromes seems to be caused by heparin induced release of PAPP-A from the arterial wall and not due to excretion from vulnerable plaques.

Introduction

In 2001 Bayes-Genis et al. demonstrated that circulating pregnancy associated plasma protein-A (PAPP-A) was significantly higher in patients with both myocardial infarction and unstable angina pectoris than in patients with stable angina pectoris [1]. Since then numerous studies have examined the role of PAPP-A in coronary artery disease [2–22]. These studies have demonstrated that circulating PAPP-A is elevated in patients with coronary artery disease and suggested a significant prognostic importance. Based on immunohistochemial analyses some research groups have suggested the site of PAPP-A synthesis to be vulnerable atherosclerotic plaques in the coronary arteries [1,23]. However, in a recent study, we were unable to confirm these immunohistochemical findings using well characterized monoclonal anti PAPP-A antibodies on atherosclerotic plaques as well as on thrombus material isolated during percutaneous coronary intervention [24]. It has to be emphasized that during these experiments a strong and highly specific staining reaction of the cytoplasm of the syncytiotrophoblast in term placental tissue was seen [24]. Our findings led to the hypothesis: that PAPP-A in acute coronary syndrome may be derived from a site distant from the ischemic cardiac tissue.

The aim of the present study was to pursue this hypothesis by (i) expansion of our data on immunohistochemical analysis of atherosclerotic plaques and thrombi and to analyse serum concentrations of PAPP-A *in vivo* before, at and after the vulnerable plaque formation, (ii) analysis of circulating PAPP-A in patients with non-atherosclerotic myocardial infarction, (iii) examination of circulating PAPP-A in patients with extra-cardiac ischemia and (iv) analysis of whether the circulating PAPP-A in acute coronary syndromes is related to medical treatment.

Method

Ethics

The trial complied with the Declaration of Helsinki. Ethical approval was given by the local ethics committee and The Danish Data Protection Agency.

Serum samples

Serum samples for PAPP-A quantification were obtained from the following groups of patients and volunteers:

Abbreviation: PAPP-A, Pregnancy associated plasma protein-A.

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Patient group 1

Patients in group 1 were patients screened for inclusion into a previous published study of the role of PAPP-A in patients admitted with ST-segment elevation myocardial infarction, who were excluded due to normal coronary angiography and the presence of an alternative diagnosis. One blood sample for analyses of PAPP-A was collected at admission. All patients received the same medical treatment as the included patients prior to primary percutaneous coronary intervention (i.e., 10,000 IU unfractionated heparin, 300 mg aspirin and 300 mg clopidogrel).

Patient group 2

Patients in group 2 were patients undergoing percutaneous transluminal septal myocardial ablation by alcohol-induced septal branch occlusion. Patients were pre-treated with 10,000 IU unfractionated heparin. Blood samples for measurement of PAPP-A were drawn before the procedure and 0.5, 1, 4 and 8 h after the procedure.

Patient group 3

Patients in group 3 were women undergoing embolisation of uterine leiomyomas. Afterwards, leiomyomas decreases gradually in size during the next 6 months. Samples were collected before treatment and days 1, 7 and 30 after the procedure. The standard medication prior to embolisation was weak analgesic (i.e., paracetamol and non steroid anti-inflammatory drugs). The patients were treated with morphine during the first 24 h after the procedure.

Patient group 4

Patients in group 4 were healthy volunteers who had a blood pressure cuff inflated to 200 mm Hg in 15 min on the right arm. Samples for measurement of PAPP-A were drawn at baseline, after deflation, and every 5 min for the following 30 min.

Patient group 5

Healthy volunteers received 10,000 IU unfractionated heparin by intravenous injection. Blood samples were drawn before and 0.5, 1, 2, 3, 4, 5 and 7 h after injection of heparin.

Patient group 6

Eight patients undergoing percutaneous coronary intervention due to acute coronary syndrome were included. One patient had non ST-segment elevation myocardial infarction and 7 patients had ST-segment elevation myocardial infarction. All patients were treated with heparin according to standard protocol. Blood samples were drawn in the coronary arteries proximal to the culprit lesion and distal to the culprit lesion. Blood samples were drawn prior to the stenting of the culprit lesion as soon as a catheter could be placed through the lesion.

Tissue samples

In order to confirm previous findings from a qualitative experiment with tissue from an autopsy of a deceased patient without cardiovascular disease eight identical macroscopic normal tissue samples from the aorta were obtained from an autopsy from another patient without cardiovascular disease. The autopsy was performed approximately 4 h prior to the experiment. Samples weighed 0.36 g (range 0.34 g–0.39 g). The tissues were incubated at 37 °C for 1 h; in isotonic saline, and unfractionated heparin, low molecular weight heparin (Tinzaparin) or fondaparinux at different concentrations in isotonic saline.

Control tissue samples (from the same autopsy) of similar size as that of the aorta were obtained from cardiac muscle, striated muscle (biceps), liver, kidney and lung (three of each). The control samples were incubated at 37 °C for 1 h with isotonic saline, unfractionated heparin and low molecular weight heparin. The total amount of fluid in all experiments was 8 mL.

Immunohistochemistry

Immunohistochemistry was performed on formalin fixed paraffin embedded tissue sections. For antigen retrieval we used both proteases (Pronase E (protease type XIV, Sigma, USA)) and heat-induced epitope retrieval (TEG-solution (10 mM Tris + 0.5 mM EGTA, pH 9.0) and TRS (Target Retrieval Solution, DakoCytomation A/S, Copenhagen, Denmark)). The procedure has been described in details previously [24]. Atherosclerotic plaques were collected as a part of studies previously published [25–27]. Control samples were stored tissue obtained from the Department of Pathology, Odense University Hospital, Denmark.

The plaques originated from 22 subjects of whom 11 died from coronary atherosclerosis and 11 died from other causes. The cause of death was classified as coronary atherosclerotic death if no extra-cardiac cause of death was found and a thrombus was superimposed on an atherosclerotic plaque (n = 6) or at least one epicardial coronary artery had >75% cross-sectional luminal narrowing by an atherosclerotic plaque (n = 5)[28].

The applied primary antibodies were monoclonal anti-PAPP-A with defined specificity [24]. Dilutions with different amounts of antibody were tested on the positive control, and the dilution, which gave the strongest signal, was chosen.

Formalin fixed paraffin embedded tissue sections of term placenta were used as positive control. Plaques were classified according to the classification of atherosclerosis endorsed by the American Heart Association [29].

Size chromatography

Size chromatography was performed on a 24 mL Superose ™6 column (Amersham Bioscienses) connected to an Ächta FPLC system. The flow rate was 2.5 mL/min and 0.5 mL fractions were collected. The samples (200 μ) applied were late pregnancy serum (prediluted with saline), normal human non-pregnant serum (negative control), serum from a patient undergoing percutaneous transluminal septal myocardial ablation (sample taken 0.5 h after procedure) and serum from a volunteer, who received 10,000 IU of unfractionated heparin intravenously (sample taken 0.5 h after injection).

Quantification of PAPP-A

PAPP-A in sera or incubation fluid (tissue samples) was quantified by ELISA technology based on monoclonal antibodies and validated against the original radioimmunoassay as described in details previously [24]. The monoclonal antibodies applied in the present assay react with epitopes, which are accessible in free as well as complexed PAPP-A.

The detection (quantification) threshold of the original assay was 3.7 mIU/L, whereas the detection limit of the present method, following recalibration, was calculated to be 4 mIU/L. The intra-assay coefficients of variation at 71.7 mIU/L and 10.4 mIU/L (n = 24) were 2.0% and 5.7%, respectively, and the corresponding inter-assay coefficients of variation (n = 14) were 6.4% and 8.7%. The dynamic range of the ELISA technique was from 2 to 200 mIU/L.

Results

Serum samples

Patients suspected for ST-segment elevation myocardial infarction

Of the 354 patients screened for this study 12 patients had normal smooth coronary arteries and one had atheromathosis. These patients