AGGREGATORY BEHAVIOUR OF PLATELETS INCUBATED WITH SUBCELLULAR FRACTIONS OF NORMAL AND CHAGASIC HUMAN SYNCYTIOTROPHOBLAST

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SUMMARY

The surface of human syncytiotrophoblast does not induce maternal blood platelet aggregation even though it is not an endothelium. It can be surmised that as occurs in endothelial injury the subcellular components of the syncytiotrophoblast may have pro-or antiaggregatory activity. During congenital Chagas’ disease which is associated to trophoblast lesions, platelets may play a role in the development of T. cruzi-induced placentitis. In the present work the aggregatory behaviour of normal human blood platelets was recorded after their challenging with subcellular fractions of syncytiotrophoblast isolated from normal and chagasic women. Nuclear, Mitochondrial, Microsomal and Supernatant fractions isolated from normal and chagasic syncytiotrophoblast failed to induce per se any aggregatory reaction on platelets. When samples of platelet-rich plasma (PRP) were preincubated with normal and chagasic nuclear fractions and then stimulated with collagen at threshold level (CT-PRP) an inhibition of the aggregatory response was observed. Treatment of CT-PRP with normal and chagasic mitochondrial fractions induced inhibition of platelet aggregation whereas only chagasic fraction reduced latency time. Microsomal fraction from normal placentas showed no significant effects on platelet aggregation. It is concluded that subcellular fractions of normal human syncytiotrophoblast do not exhibit any effect on platelet aggregation, whereas those subcellular fractions enriched in intracellular membrane components isolated from chagasic placentas inhibit platelet aggregation.

KEY WORDS: Human congenital Chagas’ disease; Human platelet aggregation; Trypanosoma cruzi; Chagas’ disease; Placenta.

INTRODUCTION

Platelet aggregation occurs when contact with the subendothelium surface is made. They also promptly react when incubated with subcellular fractions of certain organs and tissues such as liver, myocardium, brain, skeletal muscle and kidney. Even though the luminal surface of human syncytiotrophoblast is not considered a typical endothelium, maternal platelet aggregation does not occur.

The passage of T. cruzi from the blood of an infected pregnant woman to the fetus through the placenta may produce congenital Chagas’ disease, in which widespread hemorrhagic necrosis occurs. Platelets play a role in the capture of T. cruzi from circulation. Large areas of denudated trophoblastic villi and necrotic lesions had been reported in placental tissues from chagasic women suggesting that platelet disbalance may play a role in the hemorrhagic lesions described in this infectious disease. This study was performed to determine the aggregatory response of resting and threshold stimulated human platelets challenged with subcellular fractions of normal or chagasic human syncytiotrophoblast.

MATERIAL AND METHODS

Platelets were obtained from human blood of 28 volunteers of both sexes, 20-40 years old.

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Blood was collected into 3.8% sodium citrate (9:1, v/v) and centrifugated to obtain platelet rich plasma (PRP) and platelet poor plasma (PPP). In order to detect any synergistic or inhibitory effect induced by the subcellular fractions, samples of 450μl of PRP were preincubated for 5 minutes with the corresponding subcellular fraction and then stimulated at threshold level with collagen (calf skin, Type I, Sigma Chemical Co.). The appropriate collagen concentrations were established through preliminary experiments in each volunteer as described elsewhere. Negative and positive control samples were obtained by incubating PRP with phosphate buffered saline (PBS) or collagen, respectively. Aggregation studies were performed using a single channel aggregometer (CYBORG Electrónica, Argentina). The following parameters were studied: 1. The length of the lag time, recorded from the addition of the subcellular fraction until the onset of the first sharp pen deflection. 2. The maximal transmission (scale deflection), indicator of the completion of the aggregation expressed as a percentage of aggregation. Data were evaluated by variance analysis (ANOVA). The significance level was established at p<0.05. Placentae were obtained after normal term delivery from 10 normal and 4 chagasic women. The pathological condition was diagnosed by immunofluorescence and Machado-Guerreiro tests. These women did not have clinical evidence of the disease.

Nuclear, mitochondrial, microsomal and supernatant fractions were obtained from isolated normal and chagasic syncytiotrophoblast by ultracentrifugation. Purity of the fractions was evaluated by electron microscopy as previously described.

Samples of normal and chagasic fractions suspended in PBS containing 135, 270 and 405μg/protein were added to 950μl aliquots of PRP and then stimulated or not with collagen as described above.

RESULTS

Compared with positive controls stimulated only with collagen, nuclear, mitochondrial, microsomal and supernatant fractions isolated from both normal and chagasic syncytiotrophoblast at the three concentrations studied failed to induce per se any aggregatory changes on normal resting platelet. As shown in figure 1 pretreatment with normal nuclear fraction upon CT-PRP induced inhibition of the percent of aggregation, being this effect more intense with the chagasic fraction, in comparison to controls stimulated only with collagen. Similar inhibition was recorded when normal and chagasic mitochondrial fractions were pre-added to CT-PRP samples whereas only chagasic fraction shortened the lag time period (fig. 2). Normal microsomal fraction induced an increase of the percent of aggregation of CT-PRP samples and the chagasic microsomal fraction exerted an inhibitory effect on the percent of aggregation of CT-PRP but the observed differences were on the limits of signification (fig. 3). No modifications were recorded on the lag time parameter with both fractions with respect to the positive controls. Preincubation of CT-PRP with normal and chagasic supernatant showed erratic behaviour; thus, the results were inconclusive.

DISCUSSION

Platelets seem to be important in the removal of circulating T. cruzi from the blood stream. Chronic tissue injury induced by nidation of T. cruzi in the trophoblast is accompanied by the recruitment of macrophages and leukocytes. This fact could favor the activation of lipolytic enzymes and phospholipases, which in turn trigger the arachidonate cascade with a disbalanced production of vasoactive metabolites such as the pro-aggregatory thromboxane B2 and antiaggregatory hydroxyeicosa­tetraenoic acids.

Even though the subcellular fractions were isolated from human syncytiotrophoblast devoided of connective tissue cells, the remaining activity of hydrolytic enzymes and ADP could be responsible of the shortening of the lag time induced by the pre-addition of chagasic mitochondrial fractions on CT-PRP samples in the early phase of aggregation.

Subcellular mitochondrial and nuclear fractions isolated from normal placentae induced a significant inhibition of platelet activity. This inhibition was more marked when platelets were incubated with subcellular fractions obtained from chagasic placentae. The inhibition of the aggregation observed with most of the subcellular fractions, which were more evi-
dent in chagasic placentae, having a thick basal membrane could be modulated by fibronectins and other antiaggregatory glycoproteins originated from this basal membrane. Fibronectin is a glycoprotein which inhibits the last stage of platelet aggregation but induces a shortening of the lag time, probably by the limitation of platelet-collagen interaction.

Circulating epimastigotes of T. cruzi permeate syncytiotrophoblast inducing small multifocal cytological injuries which progress until the cell disruption. These abnormalities expose intracellular membranes of human syncytiotrophoblast to the contact with both, platelets and circulating parasites, thus favoring the activation of the former. Indeed, platelets seem to be involved in the mechanisms of protection against T. cruzi since mice experimentally depleted of platelets were more susceptible to infection with T. cruzi. In our experimental conditions microsomal fractions were enriched in plasma membrane whereas other internal membranes components such as endoplasmic reticulum were present to a lesser extent as indicated by their increase of the 5' nucleotidase and alkaline phosphatase activities and electron microscope observations. It has been reported that in normal conditions the luminal border of the human syncytiotrophoblast is the main contributor of plasma membrane to the microsomal fraction. Although minor inhibitory effect of microsomal fraction on the aggregation were recorded when this fraction was isolated from chagasic women it may be concluded that those areas of the syncytiotrophoblast usually exposed to the maternal circulating platelets do not exert any detectable action on the aggregation. On the contrary, those subcellular fractions enriched in intracellular membrane components inhibit platelet aggregation. This effect was more evident in chagasic placentae suggesting that the modifications of membranes induced by T. cruzi might be the reason for the demonstrated platelet functional abnormalities and might also be involved in the pathogenesis of chagasic placentitis.
RESUMO

Comportamento agregatório das plaquetas incubadas com frações subcelulares de sinciciotrofoblasto humano normal e chagásico.

A superfície do sinciciotrofoblasto humano não induz agregação das plaquetas maternas apesar de não ser um endotélio. Lesões endoteliais propiciam o aparecimento de agregados plaquetários, o que nos levou a questionar se os componentes subcelulares do sinciciotrofoblasto também poderiam propiciar eventos semelhantes. Na doença de Chagas congênita, que está associada a lesões a nível de trofoblasto, as plaquetas poderiam desempenhar algum papel no desenvolvimento da placentite induzida pelo T. cruzi. Neste trabalho estudou-se o comportamento agregatório das plaquetas humanas normais expostas à frações subcelulares do sinciciotrofoblasto isolado de placentas de mulheres normais e chagásicas. As frações Nuclear, Mitocondrial, Microsomal e Sobrenadante isoladas do sinciciotrofoblasto normal ou chagásico não induziram por se reação agregatória de plaquetas. Quando amostras de plasma rico em plaquetas (PRP) foram pré-incubadas com fração nuclear de plaquetas normais ou chagásicas e pós-estimuladas com doses limitares de colágeno (DLC-PRP) observou-se uma inibição da resposta agregatória. O tratamento de DLC-PRP com fração Mitochondrial de trofoblasto normal e chagásico também induziu inibição da agregação plaquetária porém somente a fração chagásica diminuiu o tempo de latência. A fração Microsomal das plaquetas normais não provocou diferenças significativas na agregação plaquetária. Conclui-se que as frações subcelulares do sinciciotrofoblasto humano normal não tem ação significativa na agregação plaquetária, enquanto que a incubação com frações subcelulares de plaquetas chagásicas, enriquecidas em componentes membranosos intracelulares, induziu a inibição da agregação plaquetária.

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