STUDIES ON THE MECHANISM OF SYNTHESIS AND RELEASE OF THE PROCOAGULANT ACTIVITY FROM LEUKAEMIC CELLS

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(Received 3 April 1989; accepted in revised form 6 June 1990)

The synthesis and release of procoagulant activity (PCA) from leukaemic leucocytes was studied in an in vitro culture system stimulated by endotoxin. Puromycin, actinomycin-D, vinblastine, colchicine, dibutyryl cyclic AMP and ouabain were added to the culture system to study some of the metabolic processes of these cells in relation to synthesis and release of PCA. It was found that production of PCA is an active process and depends on new protein synthesis. The release of PCA from cells can be inhibited by vinblastine, an inhibitor of microfilament and microtubules in the cell. The optimal release of PCA occurs at pH 7.2-7.4 at 37°C and is not inhibited by the ATPase inhibitor ouabain. Dibutyryl cyclic AMP inhibits the release/synthesis of PCA.

Gram negative septicaemia and endotoxinaemia are capable of increased production and release of PCA from leukaemic cells and could contribute to the coagulation failure seen in this disease.

Key words: Procoagulant activity, Leukaemic cells, Disseminated intravascular coagulation (DIC), Endotoxin, Secretion.

LIST OF ABBREVIATIONS

PCA — Procoagulant activity.
ALL — Acute Lymphoblastic Leukaemia.
CGL — Chronic Granulocytic Leukaemia.
CLL — Chronic Lymphocytic Leukaemia.
ANLL — Acute Non-lymphocytic Leukaemia.
D-Cyclic AMP — Dibutyryl — Cyclic AMP.

INTRODUCTION

Haemostatic failure is not an uncommon complication in haematological malignancies particularly in acute promyelocytic leukaemia and in certain varieties of acute myelogenous leukaemias.1 The coagulation system in these disorders is disturbed by several mechanisms like associated thrombocytopenia, acquired deficiency of certain coagulation factors, functional defects in platelets and consumption coagulopathy. It has been postulated that due to liberation of increased amount of procoagulant material from the leukaemic leukocytes activation of blood coagulation takes place resulting in disseminated intravascular coagulation.2-6 How the leukaemic cells liberate the procoagulant material has been less well studied. It is believed that this substance is liberated due to the death of the cells during chemotherapy.7 However, DIC and the resultant bleeding in cases of acute leukaemias is very often seen independent of chemotherapy. Therefore, one of the possible mechanisms is secretion of procoagulant material by viable leukaemic cells from time to time. The metabolic processes that regulate the synthesis and release of procoagulant activity from leukaemic cells are not known.

In the present communication we tried to explore the possibility of secretion of PCA by leukaemic leukocytes by using a short term in vitro culture system with added endotoxin as a stimulus. Endotoxin was chosen as a stimulus because the role of infection and endotoxinaemia in the above processes may be important in view of frequent infections accompanying leukaemias. We tried to answer the following points in the present communication:

(i) Whether procoagulant activity (PCA) can be liberated by viable leukaemic cells in short term culture.

(ii) Whether PCA is synthesized by the leukaemic leukocytes in response to endotoxin.

(iii) Some of the metabolic processes involved in production and release of PCA from leukaemic cells.
MATERIALS AND METHODS

(a) Sample

Blood samples were collected in acid citrate dextrose (ACD) (9 parts of blood: 1 part of ACD) from 66 patients (AML-27, CGL-17, CGL in blast crisis-3, ALL-6, CLL-5) and controls (8 healthy subjects). Informed consent was taken from all individuals before venepuncture. Leucocytes were sedimented by dextrose sedimentation technique at 25°C and the cell button was washed three times with phosphate buffer saline (PBS-pH 7.4) at 4°C.

Contaminating red blood cells were lysed by hypotonic shock and white cells were washed and finally suspended in a tissue culture media RPMI-1640. Viability of leucocytes was tested by 0.1% trypan blue dye exclusion test at the beginning and at the termination of culture. About 80-90% cells were viable when they were put in the short term culture system.

(b) Short term culture

The leukaemic leucocytes (1 × 10⁸ cells ml⁻¹) were resuspended in 1 ml volume with RPMI 1640 in sterile glass tubes with caps and incubated at 37°C with and without addition of 0.1 ml of E. coli endotoxin (E. coli 026 B6) 100 μg ml⁻¹ obtained from Difco Laboratories, Detroit.

Different additives like puromycin, vinblastine, actinomycin-D or dibutyryl cyclic AMP (Sigma Chemicals, St. Louis, Missouri) were added to the culture tubes in addition to endotoxin in duplicate tubes at final concentrations mentioned under Results. The cells were cultured for 18 h at 37°C. The supernatants were separated by centrifugation at 1000 rpm and pH adjusted to 7.1-7.2 by dialysis against buffered saline.

In addition to this, in a separate series of experiments the temperature optima, pH optima and the time course of release of PCA by leukaemic leucocytes were studied in cells stimulated with endotoxin.

(c) Assay of procoagulant activity

Procoagulant activity in the supernatant and cell lysates was measured by one stage assay. Briefly this assay involves measurement of plasma recalcification time on pooled normal plasma after addition of 0.1 ml of cell supernatant or 0.1 ml cell lysate as the case may be. 0.1 ml of normal saline (NS) and 0.1 ml of tissue culture media (RPMI-1640) were added to normal pooled plasma and plasma recalcification time measured on this mixture provided the control value in the absence of any PCA.

Plasma recalcification time was done with addition of cell lysate or culture supernatant on factor VII and factor VIII deficient plasma to see whether PCA is dependent on factor VII activity or factor VIII activity. Factor VII is involved in an extrinsic pathway of blood coagulation whereas factor VIII is involved in an intrinsic pathway of blood coagulation. The culture supernatants and cell lysates were tested separately in duplicates and the mean is taken as the final reading.

The cell lysates were prepared by repeated freezing and thawing. Complete lysis was confirmed by checking the absence of intact cell under microscope. The protein content of cell lysate was measured by the Lowry method and the result was expressed in terms of cell count.

RESULTS

As is shown in Table 1, the maximum PCA is present in cells obtained from acute promyelocytic leukaemia patients and the least in the cells of lymphoid origin (i.e. in cells from ALL and CLL patients). E. coli endotoxin stimulated the synthesis of PCA which was ultimately released to the culture supernatants.

This procoagulant material is similar to other tissue thromboplastins and requires the presence of factor VII for its activity and the material (PCA) is not involved in the intrinsic pathway of blood coagulation as factor VIII deficient plasma behaves in the same way as normal pooled plasma (Table 2). The activity of PCA in the supernatants was found to be proportional to the protein content (Fig. 1) and stimulation of cells with endotoxin resulted in parallel increase in protein content as well as PCA in the media.

(a) Kinetics of release of PCA

The time course of release of PCA from leukaemic leucocytes after stimulation with endotoxin is shown in Fig. 2. PCA in the culture supernatant can be detected as early as 1 h after incubation and the activity slowly increased to its peak level by 12-18 h of incubation and the activity declined over 22-24 h of incubation.

(b) Mechanism of release of PCA

(i) Effect of pH and temperature. Optimum pH for