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Fibrinogen is chemotactic for vascular smooth muscle cells

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We studied the effect of fibrinogen on the migration of bovine aortic smooth muscle cells in culture, using a Neuro Probe 48-well micro chemotaxis chamber. Fibrinogen stimulated the migration of the cells dose-dependently at concentrations from 30 to 1000 μg/ml. A modified checkerboard analysis of the response demonstrated that the effect was largely chemotactic in nature. The present results suggest that fibrinogen may play an important role in the pathogenesis of arterial intimal thickening and atherosclerosis.

Fibrinogen; Chemotaxis; Atherosclerosis; Intimal thickening; (Smooth muscle cell)

1. INTRODUCTION

It is widely accepted that the migration of vascular smooth muscle cells (SMCs) from the media into the intima and their proliferation in the intima have an important role in the pathogenesis of atherosclerosis and diffuse intimal thickening. Ross and Glomset [1] proposed that platelet-derived growth factor (PDGF) was the main cause of the process, by stimulating the migration and proliferation of vascular SMCs. However, there is also accumulating evidence to suggest the importance of the clotting system in the initiation and progression of atherosclerosis [2-4].

In this study, we examined the effect of fibrinogen on the migration of vascular SMCs.

2. MATERIALS AND METHODS

2.1. Materials

Purified bovine fibrinogen (BP-073) was obtained from Binding Site Ltd (USA). SDS-polyacrylamide gel electrophoresis demonstrated only a single band. Fibronectin, plasminogen, factors II, VIII, and XIII, von Willebrand factor, and fibrin monomer could not be demonstrated by established criteria [5]. Other materials were purchased from the following sources: bovine serum albumin (BSA, essentially fatty acid- and globulin-free, A-0281), porcine plasmin (P-8644) and soybean trypsin inhibitor from Sigma (USA); trypsin from Gibco (USA); Dulbecco's modified Eagle's medium (DMEM) from Nissui (Japan); fetal bovine serum (FBS) from Cell Culture Laboratories (USA).

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2.2. Cell culture

Bovine fetuses were obtained from a slaughterhouse. Thoracic aorta was isolated aseptically and the inner surface was rubbed gently to remove endothelial cells. After the adventitia was removed carefully, medial SMCs were cultured using the explant method described by Ross [6]. Cells were grown in 25 cm^2 flasks (Falcon, USA) in DMEM supplemented with 10% (v/v) FBS, pH 7.4, in a CO_2 incubator under the conditions of 37°C, 95% air/5% CO_2, and humidity of 100%. The cells reached confluence within 3 weeks. SMCs were characterized morphologically with a phase-contrast microscope and a transmission electron microscope [7] and by the demonstration of actin filaments [8]. Then they were subcultured by treatment with 0.05% trypsin/0.02% EDTA and split at a 1:4 ratio. Cultures were used for the migration assays within 8 passages.

2.3. Cell migration assays

Single cell suspensions of SMCs were obtained by treatment of cultures with 0.05% trypsin/0.02% EDTA. Harvested cells were washed in serum-free DMEM supplemented with 0.5% (w/v) BSA and were resuspended in the medium. Migration assays were performed using a 48-well micro chemotaxis chamber (Neuro Probe, USA) [9]. The lower wells, containing 27 μl of the solution to be assayed, were covered with a polyvinylpyrrolidone-free polycarbonate filter (5 μm pore size), and the resuspended cells were added to the upper wells at a volume of 45 μl (2.5×10^4 cells/well). After a 6-h incubation in

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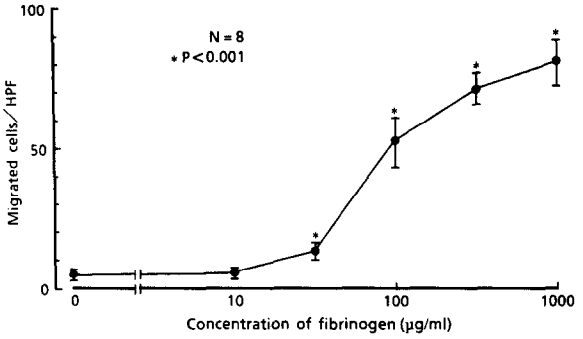

Fig.1. The effect of fibrinogen on the migration of SMCs. Results are expressed as mean ± SD.

a CO_2 incubator, the filter was removed from the chamber and non-migrated cells were scraped from the upper surface. Migrated cells were then fixed, stained with Giemsa, and counted under a high power (400×) field (HPF). 4HPFs were counted per sample and averaged.

For testing the specificity of fibrinogen-induced SMC migration, fibrinogen was used at 300 μg/ml. DMEM supplemented with 0.5% BSA was used as the medium. Dialyzed fibrinogen was obtained by dialysis overnight against DMEM at 4°C in

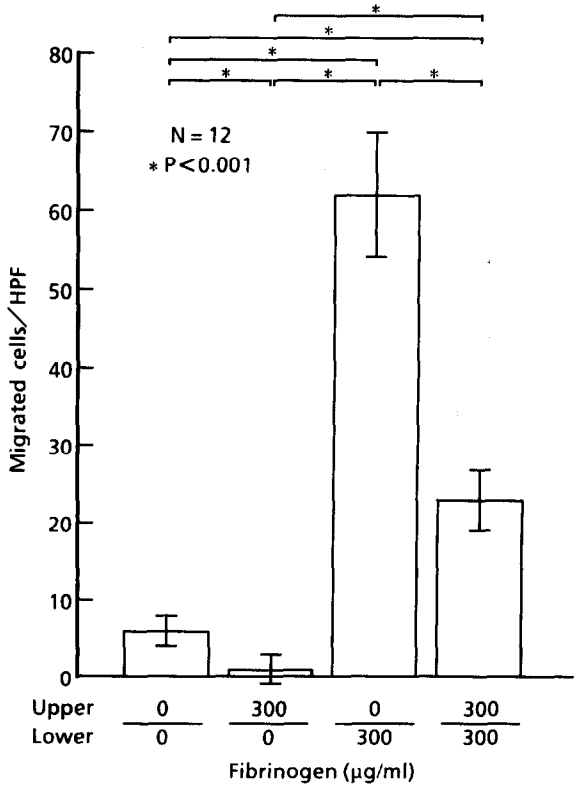

Fig.2. The effect of the gradients of fibrinogen on the migration of SMCs. Results are expressed as mean ± SD.

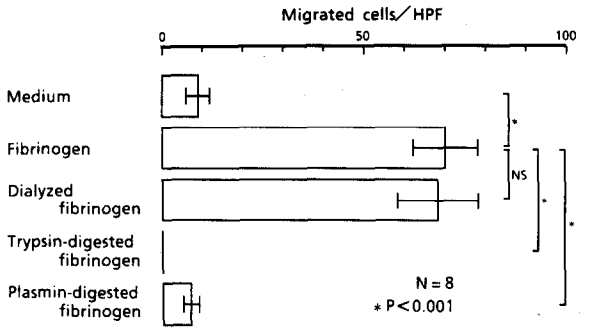

Fig.3. Specificity of the migration of SMCs induced by fibrinogen. Results are expressed as mean ± SD. NS, not significant.

Visking dialysis cellulose tubing (Niplone, Japan) with a pore size of 24 Å and used at 300 μg/ml. Digestion of fibrinogen by trypsin was performed by overnight treatment of fibrinogen with trypsin (1 mg/ml) and subsequent neutralization of trypsin with soybean trypsin inhibitor (1 mg/ml). Plasmin-digested fibrinogen was obtained by overnight treatment of fibrinogen (300 μg/ml) with plasmin (2.5 U/ml) and subsequent neutralization with soybean trypsin inhibitor (1 mg/ml).

3. RESULTS

Fibrinogen stimulated the migration of SMCs dose-dependently in the range between 30 and 1000 μg/ml (fig.1). A modified checkerboard analysis demonstrated that the response of SMCs to fibrinogen is largely chemotactic in nature (fig.2).

In order to investigate the specificity of the chemotaxis induced by fibrinogen, we performed the following experiments (fig.3). The same chemotactic effect was observed when fibrinogen was dialyzed. This effect was completely abolished by trypsin or plasmin digestion of fibrinogen. These results indicate that the induction of SMC migration by fibrinogen is really attributable to this substance and not to possible contaminating substances.

4. DISCUSSION

The presence of fibrinogen or fibrin antigen in atherosclerotic lesions has been confirmed by a number of investigators using immunospecific staining and other immunologic methods [10–14]. Smith et al. [13,14] suggested that fibrinogen might enter into the intima by means of filtration and that subsequently it could be rendered insoluble by means of thrombin [14] or transamidase [15] which are present in the lesions. Recently it has been

shown that samples from grossly normal aortas contained only fibrinogen and fibrin I, whereas fibrinogen concentration decreased and fibrin II concentration increased with increasing severity of atherosclerotic lesions [16]. It has also been reported that fibrinogen appears to be the most reliable marker of intimal thickening in small-sized arteries like temporal arteries and interlobar renal arteries because fibrinogen was the only protein which showed congruous mean values for the two different arterial types [17]. The present study suggests that infiltration of plasma fibrinogen into the intima due to increased endothelial permeability may be an initiating factor of intimal thickening and atherosclerosis, inducing the migration of medial SMCs into the intima.

It has been suggested that PDGF may be a key substance in the pathogenesis of atherosclerosis, because it is both mitogenic and chemotactic for SMCs in vitro [18]. However, it should be emphasized that PDGF is also chemotactic for polymorphonuclear leukocytes (PMNs) [19], whereas fibrinogen is not [20]. Atherosclerotic lesions usually contain monocyte/macrophages and a few lymphocytes [21], however, they seldom contain PMNs. The specificity of fibrinogen to SMCs therefore makes the hypothesis that fibrinogen plays a significant role in the pathogenesis of atherosclerosis and diffuse intimal thickening an attractive one.

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