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Growth Regulation of Fibroblasts by Thrombin, Factor XIII and Fibronectin

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Regulation der Fibroblastenproliferation durch Thrombin, Faktor XIII und Fibronectin

Zusammenfassung. Thrombin und Faktor XIII stimulieren die Fibroblastenproliferation unter gleichzeitiger intrazellulärer Zunahme von cGMP. Fibronectin hemmt dagegen die relative ^{3}H-Thymidin-Aufnahme von Fibroblasten. Neuraminidase-Inkubation der Fibroblasten schaltet die Thrombinwirkung vollständig aus, die Faktor XIII-Wirkung wird zu höheren Konzentrationen hin verschoben. Es wird diskutiert, daß Thrombin und Faktor XIII die Fibroblastenproliferation wie Gewebshormone stimulieren und zusammen mit dem hemmenden Fibronectin eine Regulation des Fibroblastenwachstums im Rahmen der Thrombusorganisation, der Wundheilung und des arteriosklerotischen Gefäßwandprozesses (Intimafibrose) bewirken.

Schlüsselwörter: Thrombin – Faktor XIII – Fibronectin – Gewebshormone – Fibroblastenproliferation – Thrombusorganisation – Wundheilung – Arteriosklerotischer Gefäßwandprozeß

Summary. The stimulation of fibroblast proliferation by thrombin and factor XIII is accompanied by an intracellular increase of cGMP. In contrast fibronectin inhibits the ^{3}H-thymidine uptake of fibroblasts. Pre-treatment of fibroblasts with neuraminidase eliminates the stimulating effect of thrombin completely and induces a shift of the optimum stimulating effect of factor XIII to higher concentrations. It is discussed that thrombin and factor XIII stimulate the proliferation of fibroblasts as growth hormones and regulate in combination with the inhibiting fibronectin the growth of fibroblasts in thrombus organization, wound healing and in the arteriosclerotic vessel wall process.

Key words: Thrombin – Factor XIII – Fibronectin – Growth hormones – Fibroblast proliferation – Thrombus organization – Wound healing – Arteriosclerotic vessel wall process

Blood coagulation, wound healing and thrombus formation are closely related phenomena including the formation of thrombin, the activation of factor XIII and the conversion of fibrinogen to fibrin. Proliferation of fibroblasts ist not only involved in the organization of thrombi and wound healing but also in the progression of arteriosclerotic vascular lesions with the result of intima fibrosis. The aim of this study was to analyze the influence of thrombin, factor XIII and fibronectin upon the growth of fibroblasts with respect to their mechanism of action.

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All experiments were performed in vitro using skin fibroblasts of guinea pigs (Pirbright; 8 to 10 weeks old). Small pieces of ear skin were gently trypsinized for 24 h. Primary cultures were obtained by seeding the resulting cell solution which consisted of epidermal cells and fibroblasts into Linbro culture dishes (FB-16 24 TC). All investigations were done with secondary cultures which were taken from primary cultures by gently trypsinization and reseeding of the cells. Secondary cultures only consisted of fibroblasts. The cell cultures were incubated for 12 h with the clotting factors and as parameters for altered growth behavior ^{3}H-thymidine uptake, number of cells per culture and cGMP (cyclo-guanosinemonophosphate) and cAMP (cyclo-adenosinemonophosphate) content per cell were determined. Experimental details were described by Christophers (1974) and by Pohl et al. (1979). Bovine thrombin (60 international units per 15 mg of the lyophilized preparation, Behringwerke AG, Marburg/Lahn), human factor XIII in a highly purified preparation (OBKH 10, Ch-B43025, lyophilized, kindly supplied by Dr. Heimburger, Behringwerke AG) and fibronectin (Op 160378, total protein 204 mg, 195 mg fibronectin per vial, that is 95% purity, kindly supplied by Dr. Heimburger, Behringwerke AG, Marburg/Lahn) were used in the concentrations mentioned below. Neuraminidase from Vibrio comma (cholerae) (1 ml=1 I.U.) was purchased from Behringwerke AG, Marburg/Lahn (RKD 04). Cyclo-AMP and cyclo-GMP were determined using radioassay kits from Amersham-Buchler, Braunschweig (code TRK. 43 and code TRK. 500).

Thrombin induced dose dependent an increase in the division potential and of ^{3}H-thymidine uptake of fibroblasts as already described (Bruhn et al. 1978; Pohl et al. 1979). Now, further experiments showed that 24 h after seeding 1 I.U./ml thrombin was required for an optimum stimulation of thymidine uptake by fibroblasts whereas 72 h after seeding 10 I.U./ml were necessary to obtain the same degree of stimulation. After incubating the fibroblasts with 10 I.U./ml thrombin for 2 h the intracellular cyclo-GMP was doubled (Fig. 1). However, no detectable change in the cellular concentration of cAMP could be measured. Treatment of fibroblasts with neuraminidase (0.02 U/ml) for 1 h eliminated the stimulating effect of thrombin on ^{3}H-TdR uptake. 0.1 U/ml factor XIII induced an optimum stimulation of fibroblast proliferation 24 h after seeding the culture cells, 72 h after seeding 3.0 U/ml were required to obtain the same degree of stimulation. 5% anti-factor XIII-subunit A-serum from the rabbit (1 ml/flask, Behringwerke AG, OTOIO4, KNO5803A) inhibited the stimulating effect of factor XIII completely. Pre-treatment of fibroblasts with 0.02 U/ml neuraminidase for one hour induced a shift in the optimum stimulating concentration of factor XIII from 0.1 U/ml to 0.3 U/ml. In addition, when fibroblasts 24 h after seeding were incubated

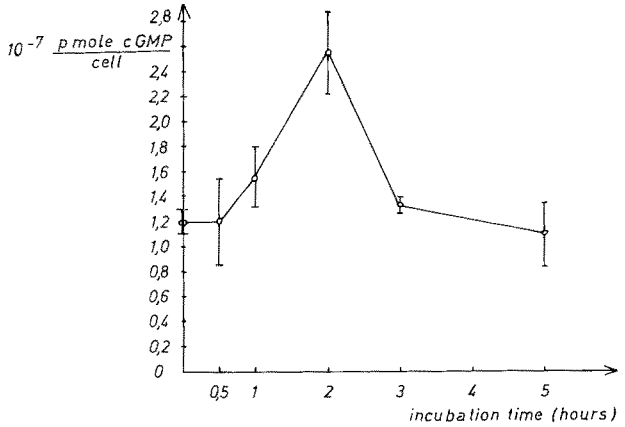

Fig. 1. Increase of cyclic guanosine-3,5-monophosphate (cGMP) in fibroblasts after an incubation with 10 IU/ml thrombin for 2 h

for 2 h with 0.1 U/ml factor XIII a nearly two-fold increase of cyclo-GMP in fibroblasts could be observed.

Fibronectin induced a concentration-dependent inhibition of ^{3}H-thymidine uptake of fibroblasts in a range from 0.03 mg/ml to 1.0 mg/ml. This effect was independent from the age of the secondary cultures and from pre-treatment with neuraminidase or trypsin.

In conclusion, thrombin and factor XIII stimulated the growth of fibroblasts dose dependent. This augmented division potential was paralleled by an increase in intracellular cyclo-GMP without affecting intracellular cyclo-AMP. These results give some explanation to former investigations of Beck et al. (1961) and Chen et al. (1975). Thus cyclo-GMP seems to be the decisive intracellular signal for fibroblast proliferation during stimulation by thrombin and factor XIII. Apparently thrombin and factor XIII have a hormone-like effect on fibroblast proliferation comparable to the effect of tissue hormones. The influence of neuraminidase could be explained hypothetically by splitting off the fragment of a receptor or by changing the conformation of this receptor. The inhibitory effect of fibronectin on fibroblast proliferation may be explained by a feed back mechanism between the fibronection of the cell surface and the DNS-synthesis of the cell nucleus. The different sensitivity of fibroblasts to thombin and factor XIII according to their cell age in secondary cultures may be due to their different positions in the cell cyclus. 24 h after seeding the majority of cells is in the pre-DNS-synthesis phase and has greatest sensitivity to a stimulating agent. After 72 h when the cell population is uniformly distributed in all positions of the cell cyclus 10- to 30-fold higher concentrations of stimulators are required. In terms of these data, thrombin and factor XIII may act as growth promotors which trigger and facilitate the cellular step into DNA-synthesis. Regarding the above data it may be understood how local liberation of thrombin and activation of factor XIII in arteriosclerotic lesions could induce proliferation of fibroblasts and as a consequence intimal fibrosis. In addition, the organization of a thrombus and wound healing are influenced significantly by proliferation of fibroblasts.

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