Plasminogen activators in alveolar bone in man

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Plasminogen activators in alveolar bone in man was studied in quenching experiments by a fibrin slide technique with addition of monospecific antibodies against tissue plasminogen activators (t-PA) and urokinase (u-PA). The plasminogen activator activity was quenched in the slides with anti-t-PA. No quenching was observed in the slides with anti-u-PA. This does not exclude the possibility that enzymatically inactive pre-urokinase is present. □ *Fibrinolytic activity; histochemistry; immunological techniques*

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The fibrinolytic system is of significance in repair and remodeling of tissues (1). It is activated by two types of plasminogen activators (PA)—that is, of tissue type (t-PA) and urokinase (u-PA). It has earlier been shown that the mucosa of the oral cavity and the alveolar bone are fibrinolytically active (2, 3). The type of PA responsible for this fibrinolytic activity has not been identified. We therefore studied the presence of t-PA and u-PA immunologically in normal alveolar bone in man.

Materials and methods

Biopsy specimens were obtained from the alveolar bone under local anesthesia (lidocaine-chloride 2% with Adrenaline, 12.5 μg/ml) from six patients referred to the Department of Oral Surgery, Malmö General Hospital, for removal of impacted lower third molars. Fibrinolytic activity was determined by a modification of the fibrin slide technique (4), as described in detail by Pandolfi et al. (5). Immunological identification of PA responsible for the fibrinolytic activity was done by a technique described by Ljungnér et al. (6).

Histochemical determination of fibrinolytic activity

In brief, sections 8 μm thick were cut in a cryostat microtome, collected on precleaned glass slides, and covered with a fibrin film rich in plasminogen. The fibrin film was obtained by clotting bovine fibrinogen solution (1%) with thrombin (Topostasin Roche, 20 NIC) (7). After a preincubation period of 30 min at 19°C the fibrin slides were incubated at 37°C in a moist chamber for 30 min and then fixed and stained. Fibrinolytic areas were reflected as clear zones of lysis, surrounded by darkly stained fibrin film.

Immunological characterization of fibrinolytic activity

Quenching of PA activity was studied with monospecific antibodies, raised in goats, against melanoma cell activator (anti-t-PA) and urokinase (anti-u-PA), and an IgG preparation from a goat before immunization. The antibodies were incorporated into the fibrin film by mixing the same concentration of the IgG fractions of anti-t-PA, anti-u-PA, and a mixture of anti-t-PA and anti-u-PA with the fibrinogen solution before the

Table 1. Quenching of fibrinolytic activity in alveolar bone sections by anti-t-PA and anti-u-PA and normal IgG

Tissue specimen	Anti-t-PA	Anti-u-PA	Anti-t-PA and anti-u-PA	IgG	Control
1	0	+++	(+)	+++	+++
2	(+)	+	0	+	++
3	0	++	0	++	++
4	0	+++	0	+++	+++
5	0	++	0	++	+(+)
6	0	++	(+)	++(+)	++(+)

+ = Microscopical punctate areas of lysis in the fibrin film in most of the sections; ++ = gross lytic areas of irregular outline and sometimes confluent; +++ = all or almost all of the fibrin film in contact with the sections lysed.

addition of thrombin. A fibrin slide with normal IgG or without IgG served as a control.

Results

High fibrinolytic activity was found in slides without antibodies and in those with normal IgG. The fibrinolytic activity was quenched in the slides with anti-t-PA added to the fibrin film. No quenching was observed in the slides with anti-u-PA, and they did not significantly differ from the controls (Table 1).

Discussion

Only a few studies are available concerning the fibrinolytic activity of bone. Björkman & Nilsson (8) showed that fibrinolytic activity was confined to highly vascularized red bone marrow, in contrast to yellow bone marrow, in which activity was absent or only slightly present. In tissue culture of fetal red bone marrow Åstedt & Pandolfi (9) found a release of plasminogen activitors into the medium. This is in agreement with the high fibrinolytic activity in the alveolar bone found in this study and also with earlier studies (2, 3) using essentially the same fibrin slide technique. The high fibrinolytic activity might thus be related to the rich vascularization of the alveolar bone. Our results also show that the fibrinolytic activity was completely quenched by t-PA. Rijken et al. (10) and Ljungnér et al. (6) have earlier shown that blood vessels contain t-PA, which is in accordance with our results.

t-PA occurs in a one-chain and a two-chain molecular form, both of them enzymatically active (11). The presence of t-PA is thus visible by the fibrin slide technique. u-PA is present in certain tissues as an inactive pre-urokinase (9), which has recently been found to be a one-chain pro-enzyme of u-PA (12, 13). Present as a pre-urokinase before secretion from the cells in its active two-chain form, u-PA is not visible by the fibrin slide technique (9). Thus, our finding of t-PA-related activity in the alveolar bone does not exclude the possibility that enzymatically inactive pre-urokinase is present.

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