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Carbonic Anhydrase Is Abundant in Fenestrated Capillaries of Cherry Hemangioma

Dedicated to the late Prof. Dr. E. H. Bárany, Uppsala

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A strong correlation has been found between carbonic anhydrase (CA) activity and fenestrations in juxtaepithelial capillaries of several tissues, including psoriatic lesions of human skin. In the present study we demonstrate that the majority of the capillaries in cherry hemangiomas are fenestrated and histochemically react CA positively. Obviously the occurrence of CA in these capillaries corresponds to the fenestrations of venous capillaries, which are numerously revealed by electron microscopy. In normal undiseased skin no capillary staining for CA was observed. Therefore in a large proportion of the capillaries of cherry hemangiomas the correlation between fenestrations and CA activity also exists. We suggest that the histochemical demonstration of CA activity might serve as a sensitive and simple marker for fenestrated capillaries in skin tissue. ***Key words: Human skin, Histochemistry, Blood vessels.***

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In previous studies capillary carbonic anhydrase (CA) has been demonstrated histochemically in a great number of tissues in several species. Concerning juxtaepithelial capillaries a strong correlation has been found between CA activity and endothelial fenestrations (1–3). Recently we could show that this correlation exists also in the intrapapillary portions of the capillary loops in psoriatic lesions (4).

In cherry hemangiomas numerous fenestrations were shown in capillaries of the tumour stroma by ultrastructural analysis (5, 6). No information is available on the presence of CA in the special situation of this vascular tumour. Therefore we investigated histochemically the presence and distribution of CA in cherry hemangioma and tried to correlate the results with ultrastructural findings.

MATERIAL AND METHODS

For this study cherry hemangiomas of 11 volunteers, aged 30 to 78 years, were examined. A ring of anesthesia was produced by intradermal injection of 1% lidocaine without epinephrine before 3- to 4-mm disks were obtained with a skin punch. It was taken care that the areas to be biopsied were not infiltrated by a previous ring of anesthesia. The samples were cut in half, fixed in 2.5% glutaraldehyde solution buffered with 0.1 M cacodylate buffer (pH 7.4) for 4 h and washed in the same buffer over night.

For *light microscopy* the specimens were embedded in water-soluble hydroxymethyl-methacrylate (JB 4, Polysciences, St. Goar, Germany, or Historesin, Reichert and Jung, Germany). One and a half μm thick semithin sections were cut perpendicular to the surface of the skin and stained for CA activity in accordance with the method of Hansson (7), as modified by Ridderstråle (8, 9).

This method requires that the sections float on the incubation medium. Therefore only sections which floated on the surface for the total incubation time were used, whereas all sections which dipped were excluded. The incubation medium contained 1.75 mM $CoSO_4$, 11.7 mM

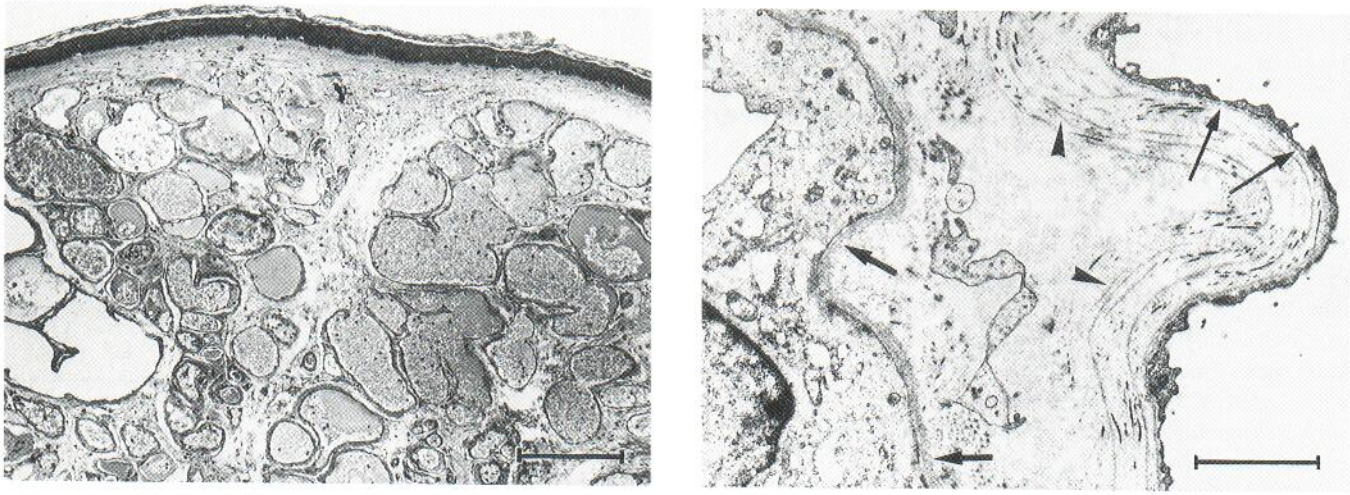

Fig. 1. (*a*) Light micrograph of skin with cherry hemangioma (hemalaun, eosin, *bar* = 200 μm). Underneath the epidermis the whole tumour stroma is filled by numerous dilated capillaries of different shape and size. (*b*) Electron micrograph of the capillary walls in cherry hemangioma (*bar* = 1 μm). There are two types of endothelial cells. On the left the capillary is lined by a high endothelium without fenestrations anchored on a single layered basal membrane (*short arrows*). The capillary on the right is lined by a very thin endothelium with fenestrations (*long arrows*) anchored on a multilayered basal membrane (*arrowheads*).

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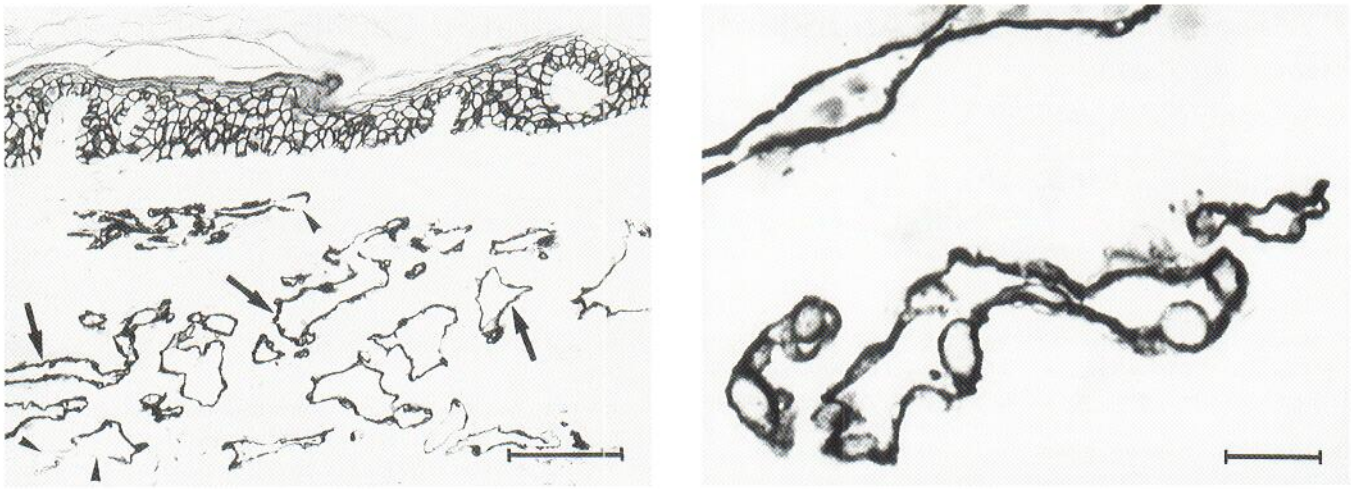

Fig. 2. Histochemical demonstration of CA in skin with cherry hemangioma (histochemical method of Hansson, 6 min of incubation). (*a*) Overview (*bar* = 25 μm). In the epidermis the cells of the stratum basale and spinosum display staining of the membranes and the cytoplasm similar to normal skin. The numerous ectatic capillaries in the tumour stroma of the dermis stain CA positive (*arrows*). In distinct regions of the capillaries CA staining is lacking (*arrowhead*). (*b*) Stained capillary endothelium at higher magnification (*bar* = 10 μm). Note that the staining is confined to the cytoplasm and membranes of the cells; the nuclei are unstained.

KH_2PO_4, 53 mM H_2SO_4 and 157 mM $NaHCO_3$ in 57 ml distilled water. After being incubated for 3, 6, 9 or 12 min, the sections were washed in 0.9% NaCl, pH 5.9, and then floated on 0.5% $(NH_4)_2S$ for 3 min. After having been washed three times for 1 min in distilled water the sections were dried on slides, occasionally counterstained with haematoxylin/eosin and then mounted in Eukitt (Kindler GmbH, Freiburg, Germany).

For *electron microscopy* the tissue samples were postfixed in 2% OsO4 and embedded in Epon in the standard manner. Ultrathin sections were stained with uranylacetate and lead citrate and then examined under the electron microscope (Zeiss 902 and 109).

RESULTS

In the tumour stroma of cherry hemangiomas histologically numerous capillaries were found, which were dilated and irregularly shaped (Fig. 1*a*). Ultrastructurally two types of capillaries were distinguishable. In most capillaries we observed a thin endothelium forming numerous fenestrations. The adjacent basal lamina was multilayered, consisting of 3 to 4 layers (Fig. 1*b*).

In a small number of capillaries we found a relatively high endothelium without any fenestrations. The endothelial cells were lacking in Weibel-Palade bodies and were anchored on a single layered basal lamina (Fig. 1*b*).

Histochemically the capillary endothelium of the 11 cherry hemangiomas investigated consistently displayed a strong staining for CA (Fig. 2*a*, *b*), indicating high CA activity. Clearly there were few capillary sections in which the reaction was faint or even lacking (Fig. 2*a*). In normal skin no CA positive capillaries were found (Fig. 3). There was also no transition zone with regard of CA staining between undiseased and tumour stroma. Therefore the extension of the tumour could easily be demarcated by the stain.

In the epidermis, the distribution of CA activity was identical in normal skin and in skin overlying cherry hemangioma (Figs. 2*a* and 3). In the stratum spinosum, the cytoplasm and all membranes of the cells were stained. The basal cells displayed CA activity in their cytoplasm and in the apical and lateral membranes. The basal part of the cell membrane facing the basal lamina did not stain. No staining was found in the cells of the stratum granulosum and corneum.

DISCUSSION

Our findings show that in cherry hemangioma two types of capillaries are distinguishable with regard to ultrastructure and CA activity. While ultrastructurally few capillaries had a high endothelium, most of the tumour capillaries were lined by a thin, highly fenestrated endothelium, anchored on a 3–4 layered basal lamina. Other investigators have characterized such vessels in human skin as venous capillaries (10, 11). In a previous paper

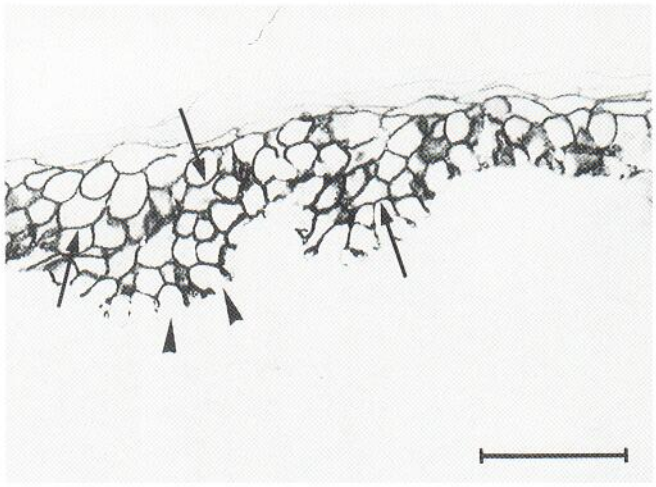

Fig. 3. Histochemical demonstration of CA activity in normal skin (chest region) of a 55-year-old man (histochemical method of Hansson, 6 min of incubation). In the epidermis all cells of the stratum germinativum display a staining of the membranes (*arrows*). In the basal cells there is staining only in the apical and lateral membranes, while the basal part of the cell membrane facing the basal lamina did not stain (*arrowheads*). In the stratum papillare the capillaries remained totally unstained (*bar* = 25 μm).

we demonstrated that in psoriatic lesions venous capillaries with a high degree of fenestrations reacted CA positively (4).

Histochemically we found a small number of CA negative capillaries in cherry hemangioma, while the vast majority of the tumour vessels displayed intense CA activity.

These findings add evidence to our previous suggestion that CA could serve as a sensitive and simple marker for fenestrated capillaries in human skin tissues. In addition, the consistent presence of the enzyme CA in endothelial cells of cherry hemangioma argues for a special degree of differentiation of these cells, as has been suggested earlier (6).

Although the function of the combined presence of fenestrations and CA in capillary endothelium is not clarified, it was suggested that it may be necessary to stabilize the pH of the endothelial cytoplasm with the aid of CA to preserve the state of fenestration (3).

Cherry hemangioma could serve as an easily available model for further investigations on this question.

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