Concentrations of endothelial-cell-stimulating angiogenesis factor, a major component of human uterine angiogenesis factor, in human and bovine embryonic tissues and decidua

C. M. Taylor¹, B. McLaughlin¹, J. B. Weiss¹ and N. G. Maroudas²

¹Wolfson Angiogenesis Unit, Department of Rheumatology, Hope Hospital, Salford M6 8HD, UK; and ²Department of Microbiology, University of Surrey, Guildford, Surrey GU2 5XH, UK

Summary. Embryonic development involves the establishment of new patterns of vascular growth in the fetus and within the lining of the womb. A factor, human uterine angiogenesis factor, has been purified from the decidua and stimulates the growth of blood vessels in collagen sponge implants and in the chick chorioallantoic membrane. Evidence is presented that suggests that a major active component of human uterine angiogenesis factor is an activator of latent matrix metalloproteinases, of low M_r , called endothelial-cell-stimulating angiogenesis factor and that this factor is present in substantial quantities in a number of embryonic tissues.

Keywords: angiogenesis; growth factor; collagenase; endothelial-cell-stimulating angiogenesis factor; human uterine angiogenesis factor

Introduction

Embryological development involves the establishment of two new networks of blood vessels, one produced by the embryo itself and one arising within the lining of the womb (decidua) in response to the growing demands of the developing fetus. Growth factors are known to play a critical role in the control of embryological development (Mercola & Stiles, 1988) and the presence of angiogenic activity in fetal tissues has been demonstrated (Burgos, 1983; Gospodarowicz *et al.*, 1985) and ascribed to fibroblast growth factors. Human uterine angiogenesis factor (HUAf), an extract derived from decidua obtained from first trimester legal terminations of pregnancy, induced neovascularization of the chorioallantoic membrane (Maroudas *et al.*, 1987) and vascularization of collagen sponge implants (Lindenbaum *et al.*, 1989). The active components of this extract are known to be of M_r 30 000 or less and one has been identified as fibroblast growth factor (FGF) (Lindenbaum *et al.*, 1989).

Endothelial-cell-stimulating angiogenesis factor (ESAF) is a nonprotein, microvessel, cell-specific mitogen of low M_r , which is capable of activating latent matrix metalloproteinases by dissociating the complexes formed with tissue inhibitor of metalloproteinases (TIMP) and also of activating promatrix metalloproteinases (McLaughlin *et al.*, 1991). This factor occurs in high concentrations where angiogenesis is occurring, such as in conditions of proliferative retinopathy (Taylor *et al.*, 1986, 1989) or in the epiphyseal growth plate (Brown *et al.*, 1987). Significant synergism exists between ESAF and FGF in the stimulation of cultured microvessel endothelial cells (Odedra & Weiss, 1987) and tissues rich in FGFs tend to contain high concentrations of ESAF (Taylor *et al.*, 1988). In this study we demonstrated that ESAF, like FGF, can be extracted from fetal tissues and decidua and is a constituent of HUAf.

Materials and Methods

Extraction of ESAF from decidua and fetal tissues

Decidua tissue was obtained from human pregnancies legally terminated during the first trimester and the material was pooled. Fetal bovine tissues were obtained from the local abattoir, gestational age being determined by the method of Bogart (1959). Lyophilized decidua were homogenized for 15 s at 4°C in NH_4HCO_3 buffer (20 ml, 50 mmol/l, pH 7.9) containing 2 mol MgCl_2 /l to remove bound ESAF from its carrier protein. Samples were centrifuged at 20 000 *g* (1 h, 4°C). The supernatants were assayed for protein (Lowry *et al.*, 1951) prior to diafiltration on a YM5 filter membrane (M_r 5000 exclusion limit; Amicon Ltd, Stonehouse, Glos, UK) with three volumes of bicarbonate buffer. The diafiltrates containing the ESAF were reduced by rotary evaporation to a volume of 10 ml and applied to an octadecyl silica column (Analytichem, Harbor City, CA, USA). Bound angiogenic material of low M_r was eluted with stepped gradient methanol and assayed. One sample from mixed supernatants was put through the complex purification procedure using high pressure liquid chromatography (HPLC) and the position of the active peak was compared with other known preparations of ESAF.

Extraction of HUAF from decidua

After homogenization of pooled human decidua in water, precipitation with 3% glacial acetic acid and rapid neutralization with NH_4OH , the crude extract was centrifuged and the supernatant filtered through a membrane cartridge (M_r 30 000 exclusion limit; Millipore Ltd, London, UK). The filtrate was then reduced to 10 ml by rotary evaporation and stored at -20°C .

Extraction of ESAF from HUAF

The 10 ml fraction of HUAF was diluted with an equal volume of NH_4HCO_3 buffer (20 ml, 50 mmol/l, pH 7.9) containing 2 mol MgCl_2 /l to dissociate bound ESAF and diafiltered on a YM5 membrane with three volumes of bicarbonate buffer. The diafiltrate was reduced to 10 ml by rotary evaporation and the ESAF was isolated as described. The protein content of the HUAF fraction was determined prior to diafiltration.

Assay of ESAF using activation of latent collagenase

Samples were assayed for their ability to activate latent collagenase according to the method of Weiss *et al.* (1983). Briefly, 75 μl of the test extract with 25 μl procollagenase was added to 50 μg collagen substrate, in 25 μl of 0.05 mol Tris-HCl buffer/l at pH 7.6, containing 0.2 mol NaCl/l and 0.01 mol CaCl_2 /l, which had been allowed to gel at 37°C for 30 min. The volume in the assay tube was made up to 300 μl and the tubes were incubated at 35°C for 4 h. At this time, 150 μl NaCl (6 mol/l) was added to precipitate any undigested, but solubilized, collagen and the tubes were allowed to stand at room temperature for 30 min before centrifugation at 1700 *g* for 30 min. Then, 200 μl of supernatant was added to 5 ml scintillation cocktail and the radioactivity was measured (in c.p.m.). Clostridiopeptidase was used to determine the total radioactivity in 50 μg collagen. Results are expressed as units of ESAF/mg protein in the supernatant, where one unit of ESAF will activate sufficient latent collagenase to degrade 1 μg collagen/h. All assays were performed in duplicate. Linearity for this method is good up to 6 units of ESAF/ml. Higher values were diluted accordingly.

Comparison of ESAF from decidua and HUAF with ESAF from other sources

A single sample (5 ml) of ESAF extract from HUAF was purified by HPLC and its elution profile was compared with that from bovine and human pineal glands, synovial fluid and serum.

Angiogenic activity *in vivo* of ESAF from decidua and HUAF

ESAF purified by HPLC was applied to a chick yolk sac membrane to confirm angiogenic activity.

Results

Both bovine and human decidua contained concentrations of ESAF comparable to those in other highly vascular tissues, such as brain and kidney (Fig. 1), but contained considerably less than the pineal gland (Taylor *et al.*, 1988). Extraction of ESAF from HUAF increased its specific activity significantly, giving 1.6 ± 0.28 units of ESAF/mg protein compared with 0.2 ± 0.04 units of ESAF/mg protein. ESAF extracted from HUAF was identical to ESAF isolated from other sources

in terms of its behaviour on HPLC. After purification, the active fraction eluted at 8 min from the final reverse-phase HPLC column, which is a time identical to that of ESAF purified from all other tissue sources (Fig. 2). ESAF from decidua and HUAF gave a typical spoke-wheel response on the chick yolk sac membrane (not shown) confirming its property as an angiogenic factor.

Fig. 1. Concentrations of endothelial-cell-stimulating angiogenesis factor (ESAF) in (a) bovine decidua, kidney and brain (average of three separate animal assays) and (b) pooled human samples of decidua, kidney and brain and of extracts of human uterine angiogenesis factor (HUAF; average of triplicate assays). Bars indicate SEM.

Fig. 2. Absorbance profile of endothelial-cell-stimulating angiogenesis factor (ESAF) from human uterine angiogenesis factor with final solvent system on high pressure liquid chromatography. This profile is identical to that of ESAF from all other tissue sources tested.

Fig. 3. Concentrations of endothelial-cell-stimulating angiogenesis factor (ESAF) in developing bovine tissues in relation to gestational age of the fetus: (a) brain, (b) growth plate and (c) diaphysis.

Extraction of ESAF from fetal bovine tissues showed a decline in concentration with increasing gestational age in the brain, diaphysis and epiphyseal growth plate (Fig. 3).

Discussion

Fibroblast growth factor is a major active component of HUAF (Lindenbaum *et al.*, 1989). We now show that ESAF is another major component of HUAF, suggesting that these two angiogenic molecules are co-distributing. In decidua a dramatic increase in the density and number of blood vessels occurs during the first trimester. The co-occurrence of ESAF and FGFs might be expected to direct the mitogenic activity of these latter factors towards the stimulation of blood vessel growth, as synergism has been reported between ESAF and basic FGF in the stimulation of microvessel endothelial cell growth *in vitro* (Odedra & Weiss, 1987); the same synergistic effect has been observed *in vivo* on the chick yolk sac membrane (Odedra & Weiss, 1991). Co-distribution of ESAF and FGF occurs in other situations; the tumour angiogenesis factor described by Folkman *et al.* (1971) is now known to contain FGFs and ESAF as its active components (Weiss *et al.*, 1979; Klagsbrun *et al.*, 1986). Neither ESAF nor FGF alone has as great an activity *in vivo* as a mixture of the two (Odedra & Weiss, 1991) and comparable results have been found *in vitro* using cultured microvessel endothelial cells (Odedra & Weiss, 1987). Similarly, we have observed that HUAF has a greater activity on the chick yolk sac membrane than either ESAF or FGF alone (J. B. Weiss, C. M. Taylor & R. Odedra, unpublished observation).

The high concentrations of ESAF detected in first-trimester human and bovine decidua may reflect the intense vascularization that occurs during this period and this is confirmed in the three fetal bovine tissues investigated, in which concentrations of ESAF declined after the first trimester (Fig. 3). ESAF may have a role in changes that occur in the cervix at parturition. Studies by Raynes *et al.* (1988a) suggest that collagenase activity in the cervix is low and does not increase during pregnancy. This may be due, in part, to the presence of substantial and increasing quantities of TIMP in the cervix (Raynes *et al.*, 1988b) causing most of any collagenase present to be in the form of a TIMP–collagenase complex. Such collagenase cannot be activated by aminophenylmercuric acetate and so is effectively silent in any assay system. That concentrations of collagenase in human cervix and lower uterine segment increase approximately tenfold during labour (Rajabi *et al.*, 1988) suggests either a rapid increase in the synthesis of collagenase at the expense of its inhibitor or the activation of indigenous collagenase complexes. ESAF is the only biological agent shown to be capable of dissociating this complex and releasing active collagenase molecules (McLaughlin *et al.*, 1991). It is therefore possible that ESAF is involved in cervical changes during labour because of its ability to activate complexed collagenase in the extracellular matrix.

References

- Bogart, R. (1959) *Improvements of Livestock*. Macmillan Press, NY.
- Brown, R.A., Taylor, C.M., McLaughlin, B., McFarland, C.D., Weiss, J.B. & Ali, S.Y. (1987) Identification of an angiogenic procollagenase activator from epiphyseal growth plate cartilage and mineralising chondrocytes in culture. *Bone and Mineral* **3**, 143–158.
- Burgos, H. (1983) Angiogenic factors from human placenta. *European Journal of Clinical Investigation* **13**, 289–293.
- Folkman, J., Merler, E., Abernathy, C. & Williams, G. (1971) Isolation of a tumour factor responsible for angiogenesis. *Journal of Experimental Medicine* **133**, 275–288.
- Gospodarowicz, D., Cheng, J., Lui, G. M., Lund, D., Crum, R. & Klagsbrun, M. (1985) Fibroblast growth factor in human placenta. *Biochemical and Biophysical Research Communications* **128**, 554–560.
- Klagsbrun, M., Sasse, J., Sullivan, R. & Smith, J. A. (1986) Human tumour cells synthesize an endothelial cell growth factor that is structurally related to basic FGF. *Proceedings of the National Academy of Science (USA)* **83**, 2448–2452.
- Lindenbaum, E.S., Kaufman, T., Beach, D. & Maroudas, N.G. (1989) Uterine angiogenic factor induces vascularisation of collagen sponges in guinea pigs. *Burns* **15**, 225–229.
- Lowry, O.H., Rosenbrough, M.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with a folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.
- McLaughlin, B., Cawston, T. & Weiss, J.B. (1991) Activation of the matrix metalloproteinase inhibitor complex by a low molecular weight angiogenic factor. *Biochimica et Biophysica Acta* **1073**, 295–298.
- Maroudas, N.G., Fuchs, A., Lindenbaum, E.S. (1987) The non-tumour perspective geometry in growth control. In *Ocular Circulation and Neovascularisation*, Eds D. BenEzra and J. Ryan. J. Martinus Nijhoff, Dordrecht.
- Mercola, M. & Stiles, C.D. (1988) Growth factor superfamilies and mammalian embryogenesis. *Development* **102**, 451–461.

- Odedra, R. & Weiss, J.B. (1987) A synergistic effect on microvessel endothelial cell proliferation between fibroblast growth factor and ESAF. *Biochemical and Biophysical Research Communications* **143**, 947–953.
- Odedra, R. & Weiss, J.B. (1991) Low molecular weight angiogenic factors. *Pharmacology and Therapeutics* **49**, 111–124.
- Rajabi, M.R., Dean, D.D., Beydoun, S.N. & Woessner, J.F. (1988) Elevated tissue levels of collagenase during dilation of uterine cervix in human parturition. *American Journal of Obstetrics Gynaecology* **159**, 971–976.
- Raynes, J.G., Anderson, J.C., Fitzpatrick, R.J. & Dobson, H. (1988a) Increased collagenase activity is not detectable in cervical softening in the ewe. *Collagen and Related Research* **8**, 461–469.
- Raynes, J.G., Clarke, F.A., Anderson, J.C., Fitzpatrick, R.J. & Dobson, H. (1988b) Collagenase inhibitor concentration in cultured cervical tissue of sheep is increased in late pregnancy. *Journal of Reproduction & Fertility* **83**, 893–900.
- Taylor, C.M., Weiss, J.B., Kissun, R.D. & Garner, A. (1986) Effect of oxygen tension on the quantities of procollagenase activating angiogenic factor present in the developing kitten retina. *British Journal of Ophthalmology* **70**, 162–165.
- Taylor, C.M., McLaughlin, B., Weiss, J. B. & Smith, I. (1988) Bovine and human pineal glands contain substantial quantities of endothelial cell stimulating angiogenesis factor. *Journal of Neural Transmission* **71**, 79–84.
- Taylor, C.M., Kissun, R.D., Schor, A.M., McLeod, D., Garner, A. & Weiss, J.B. (1989) Endothelial cell stimulating angiogenesis factor in vitreous from extraretinal neovascularisation. *Investigative Ophthalmology and Visual Science* **30**, 2174–2178.
- Weiss, J.B., Brown, R.A., Kumar, S. & Phillips, P. (1979) An angiogenic factor isolated from tumours: a potent low molecular weight compound. *British Journal of Cancer* **40**, 493–496.
- Weiss, J.B., Hill, C.R., Davis, R.J., McLaughlin, B., Sedowofia, K.A. & Brown, R.A. (1983) Activation of a procollagenase by low molecular weight angiogenesis factor. *Bioscience Reports* **3**, 171–177.

Received 24 January 1991