Vol. 153, No. 1, 1988
May 31, 1988

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 347-352

ANGIOGENIC ACTIVITY OF ADIPOSE TISSUE

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Received April 14, 1988

Adipose tissue has been used to promote wound healing and to revascularize ischemic myocardium. We explored whether fat from various sources was angiogenic in the cornea. Rabbit subcutaneous and omental fat induced grossly visible neovascularization of all rabbit corneas studied, and at a similar rate and intensity. Neovascularization was not observed in any cornea following control implantation of liver or muscle. Neovascularization was blocked in all rabbits in which indomethacin was administered orally 3 days before implantation of fat and continued following implantation, suggesting that prostaglandins are associated with fat induced angiogenesis.

Fat has long been utilized to promote wound healing. Ball (1) quoted Motley who "when describing the repulse of the Spaniards at Ostend and Antwerp in 1585, and the terrible slaughter consequent, thereon, has this to say: 'The Dutch surgeons sallied forth in strength after each encounter and brought in great bags filled with human fat, which was then esteemed the sovereignest remedy in the world for wounds and diseases.'" Surgeons have long used omentum to promote revascularization and healing of the intestines. In addition, over 50 years ago Beck described the development of anastomoses when omentum was sutured to the myocardium (2). Vineberg also demonstrated the ability of the free omentum, detached from its blood supply, to establish a capillary circulation with the myocardium (3). Ischemia of the donor bronchus resulting in dehiscence of the bronchial anastomosis has been a

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0006-291X/88 $1.50

major complication limiting lung transplantation. The omental pedicle graft is now being used to nourish the bronchus in human lung transplantation (4).

These clinical experiences noted above suggest that the improved healing induced by omental fat is the result of new blood vessel formation. Culture medium conditioned by adipoyctes differentiated from 3T3 cells is angiogenic in the chick chorioallantoic membrane (5). It has been reported that a chloroform-methanol extract made from homogenized cat omentum induces neovascularization of the rabbit cornea but that a similar preparation of subcutaneous fat does not (6). These observations raise basic questions regarding the differential effect of omental fat versus subcutaneous fat upon new blood vessel formation. We have therefore examined the angiogenicity of omental versus subcutaneous fat, using whole tissue implanted in the avascular rabbit cornea. In addition, since several lines of evidence suggest that PGE_1 and PGE_2 may play an important role in angiogenesis (7-9) we have studied the effect of blocking prostaglandin synthesis on this process.

Materials and Methods

New Zealand white rabbits (2-3 months, 3 kg) were anesthetized with intravenous pentobarbital (3-4 ml). With sterile technique, small samples of rabbit omental fat and liver were obtained through a midline abdominal incision; skeletal muscle was taken from the quadriceps and subcutaneous fat from the inguinal region. The tissue samples were bathed in normal, sterile saline at room temperature until implanted in the intra-corneal pocket of the same rabbit within 10 minutes by the method of Gimbrone et al. (10). In the first series of 7 rabbits, a sample of subcutaneous fat was placed in the corneal pocket of one eye and omental fat in the other. In the subsequent series of 10 rabbits omental fat was placed in each eye. In a control series of 5 rabbits, a sample of liver was placed in the corneal pocket of one eye and skeletal muscle in the other.

Oral indomethacin (8 mg/kg) in the drinking water was administered to 6 rabbits daily starting 3 days before omental fat was placed in the corneal pocket of each eye. Indomethacin was continued for 7 more days in half of the rabbits; in the other 3 no further indomethacin was administered after fat implantation. PGE_2 was measured in 24 hour urines collected over dry ice. Urine collection was initiated 2 days prior to administration of indomethacin. The urines were frozen and then thawed prior to performing the assay utilizing a commercially available (New England Nuclear) I^{125} radioimmunoassay kit with a detection limit for PGE_2 as low as 9 pg/ml of urine.

Corneas were examined every 2 days with a slit-lamp stereomicroscope, and the growth rate of new vessels was measured with an ocular micrometer at x 10 magnification (accuracy ± 0.1 mm). The

content of the intra-corneal pocket and time of implantation were unknown to the examiner. For histologic studies, the rabbits were sacrificed with intravenous pentobarbital. The corneas were fixed in situ with glutaraldehyde injected into the anterior chamber and by dripping fixative on the surface of the cornea. The excised corneas were post-fixed with glutaraldehyde, embedded in plastic, and sectioned for light microscopy.

Results

The response of the cornea to implanted rabbit subcutaneous and omental fat was compared in 7 rabbits. Neovascularization of the corneas occurred in all 7 rabbits at a similar rate and intensity in both eyes. Increased numbers of limbal vessels in the region of the implant were seen at 2 to 4 days. At 4 to 6 days capillary sprouts from the limbal vessels closest to the implant extended 0.1-0.2 mm into the cornea. By 10 to 14 days capillary growth had reached the fat in the corneal pocket (Fig. 1). The length of these new vessels exceeded 2-3mm. No significant difference was observed in the rate of growth or final density of the vessels grown in response to the two different types of fat. In the second series of experiments, neovascularization was observed in all 10 rabbits following implantation of omental fat in

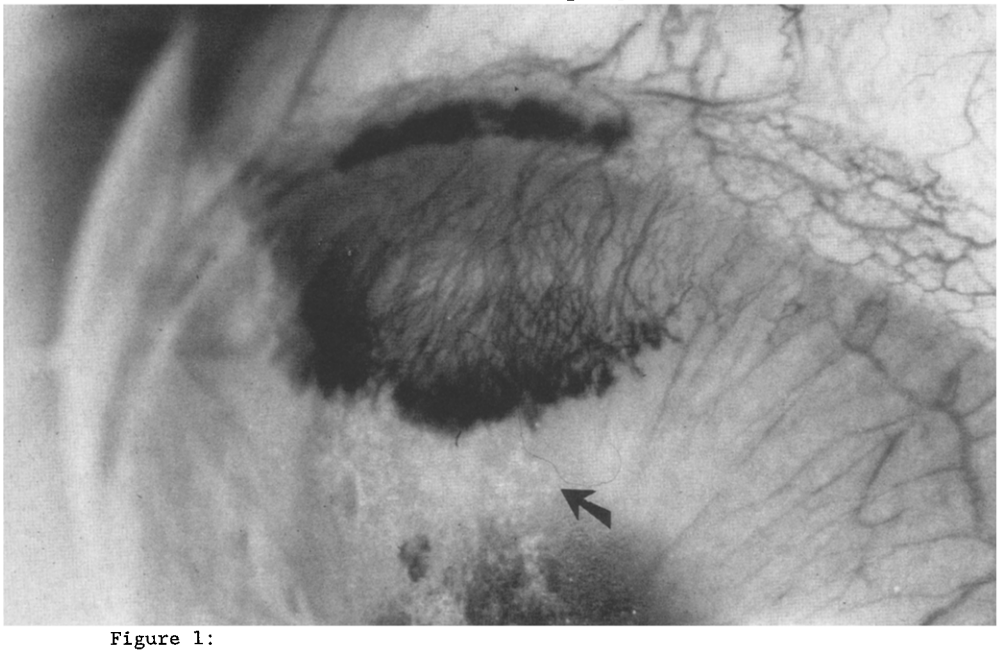

Figure 1:

Neovascularization of the rabbit cornea 10 days following implantation of rabbit omental fat. The arrow points to the fat implanted in the corneal pocket. The photograph was taken with a Zeiss slit-lamp stereomicroscope at x 10 magnification.

both eyes. The rate and intensity of neovascularization was similar in each eye, and comparable to the results in the first series of 7 rabbits. Two rabbits were followed for a total of 14 weeks. After 8 weeks, the new vessels in the cornea began to decrease in number. However, a few long thin vessels reaching to the fat in the intra-corneal pocket (over 2mm in length) persisted until the rabbits were sacrificed 14 weeks after implantation. Histologic examination of the corneas that had fat implanted confirmed the slit-lamp observations of a very rich proliferation of new vessels.

Since evidence has been presented that prostaglandins may be involved in angiogenesis the cyclooxygenase inhibitor indomethacin was administered 3 days before and continued for 7 days following implantation of fat. Indomethacin completely blocked neovascularization in 3 rabbits and was associated with a fall in the 24 hour urine content of PGE_2 from a mean baseline value of 18,200 pg to nondetectable levels. Treatment with indomethacin prior to, but not following, the implantation of fat had no noticeable effect on neovascularization in any rabbit; capillaries proliferated at a rate similar to the initial groups studied without indomethacin.

In a control series of 5 rabbits, neither muscle or liver were angiogenic, a striking difference from the previous observations in the earlier rabbit corneas implanted with fat. Table 1 summarizes the vascular response of the rabbit corneas to the tissues studied.

Discussion

Much of our understanding of angiogenesis comes from studies of neovascularization induced by tumors. Tumor growth is dependent upon an adequate blood supply, which is provided by the growth of new blood vessels from the surrounding tissue (11). However, non-malignant cells have also been shown to be capable of inducing angiogenesis. Activated macrophages and T-lymphocytes have been found to release soluble angiogenic substances (12,13). Some normal tissues have also been

TABLE I

VASCULAR RESPONSE OF THE RABBIT CORNEA TO IMPLANTED TISSUE
(examined by slit-lamp stereomicroscope)

Tissue Studied	Corneas with Neovascularization/Total
Subcutaneous fat (rabbit)	7/7
Omental fat (rabbit)	27/27
Omental fat (rabbit) with systemic indomethacin prior to and continued following implantation	0/6
Omental fat (rabbit) with systemic indomethacin only prior to implantation	6/6
Liver (rabbit)	0/5
Muscle (rabbit)	0/5

reported to show angiogenic activity, including retina, skin, salivary gland, corpus luteum, testicle and kidney (14).

The results of the present study demonstrate that both omental and subcutaneous fat stimulate an intense neovascularization of the cornea. This is in contrast to a recent publication that a chloroform-methanol extract from homogenized cat omentum induced neovascularization of the rabbit cornea, but a similar preparation of subcutaneous fat did not (6). It has also been previously reported that mouse adipose tissue did not induce neovascularization utilizing the anterior chamber preparation of the rabbit eye (14).

The ability of the indomethacin to block fat-induced angiogenesis in the cornea suggests that an arachidonic acid derivative may be associated with angiogenesis. The suppression of angiogenic activity by continued indomethacin administration, but not by pretreatment alone, suggests that prostaglandin production is essential for fat angiogenesis. This is in agreement with the demonstration by others that PGE_1 and PGE_2 may induce corneal neovascularization (7) and play a role in tumor angiogenesis (8). Moreover, treatment of 3T3 derived adipocytes with indomethacin greatly reduces their ability to stimulate angiogenesis in the chick chorioallantoic membrane (9).

Although adipose tissue was utilized over 400 years ago to promote wound healing, our knowledge of its healing action is still to be

clarified. It is of interest that despite the extensive advances in transplant immunology, a key to the recent success of lung transplantation is the application of omentum to the bronchial anastomosis. Over 50 years ago Von Euler discovered prostaglandins, but only recently has the angiogenic activity of prostaglandins begun to be studied. Our results indicate that transplanted fat is angiogenic, that this activity is not limited to the omentum, and that prostaglandins are involved. We are now examining the angiogenic activity of human epicardial fat and its possible role in the neovascularization of the coronary artery (15).

Acknowledgements

We are indebted to Richard R. Pintal, Ronald E. Cotter, and Deborah Stark for expert technical assistance and Birthe Creutz for preparation of the manuscript.

This work was supported in part by grant number HL31632 from the NIH, a grant from Smith Kline and Beckman Corporation, a gift from RJR Nabisco, and by a grant to Harvard University from the Monsanto Co.

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