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Effect of a Static Magnetic Field on the Growth Rate and *in Vitro* Angiogenesis of Endothelial Cells

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UDC 611.018.74:615.847.8

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 1, pp. 106-108, January, 1994
Original article submitted September 2, 1993

It is shown that a static magnetic field accelerates the growth rate of endothelial cells from the bovine pulmonary artery, but has no effect on the attachment and growth of cells from the human umbilical vein. A static magnetic field markedly stimulates the differentiation of endotheliocytes from the human umbilical vein to capillary-like structures.

Key Words: *static magnetic field; endothelial cells; angiogenesis*

There are some data on the effect of a strong magnetic field on the function and morphology of various cells. Exposure to a static magnetic field (SMF) has been shown to inhibit the growth and to increase the number of chromosomal aberrations in human lymphocytes [4]. Under similar conditions no changes were observed in the growth rate and morphology of WI-38 cells and human skin fibroblasts [7]. However, SMF was reported to accelerate the growth of mouse pulmonary fibroblasts [12]. Pulsed electromagnetic fields are able to stimulate osteogenesis [5], DNA synthesis [9], and cell proliferation [13], and to affect the production of extracellular matrix components in various culture systems [11].

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Endothelial cells (EC) are at present among the best studied cultured cells. These cells are responsible for athrombogenicity of the vascular bed, and are metabolically active [8]; moreover, they possess the capacity for differentiation in culture (*in vitro* angiogenesis) [10]. Endothelium is known to play an active and important role in the interaction with blood leukocytes, as well as in acute and chronic inflammation [8]. Numerous functions of EC allow for using these cells as a convenient model for studying the effects of various agents. However, little is known about the effect of SMF on EC proliferation.

The purpose of the present study was to investigate the effect of SMF on the growth and differentiation of cultured EC from human umbilical vein (HUV) and bovine pulmonary artery.

MATERIALS AND METHODS

EC isolated from HUV by dispase digestion [1] were cultured at 37°C in 5% a CO_2 atmosphere

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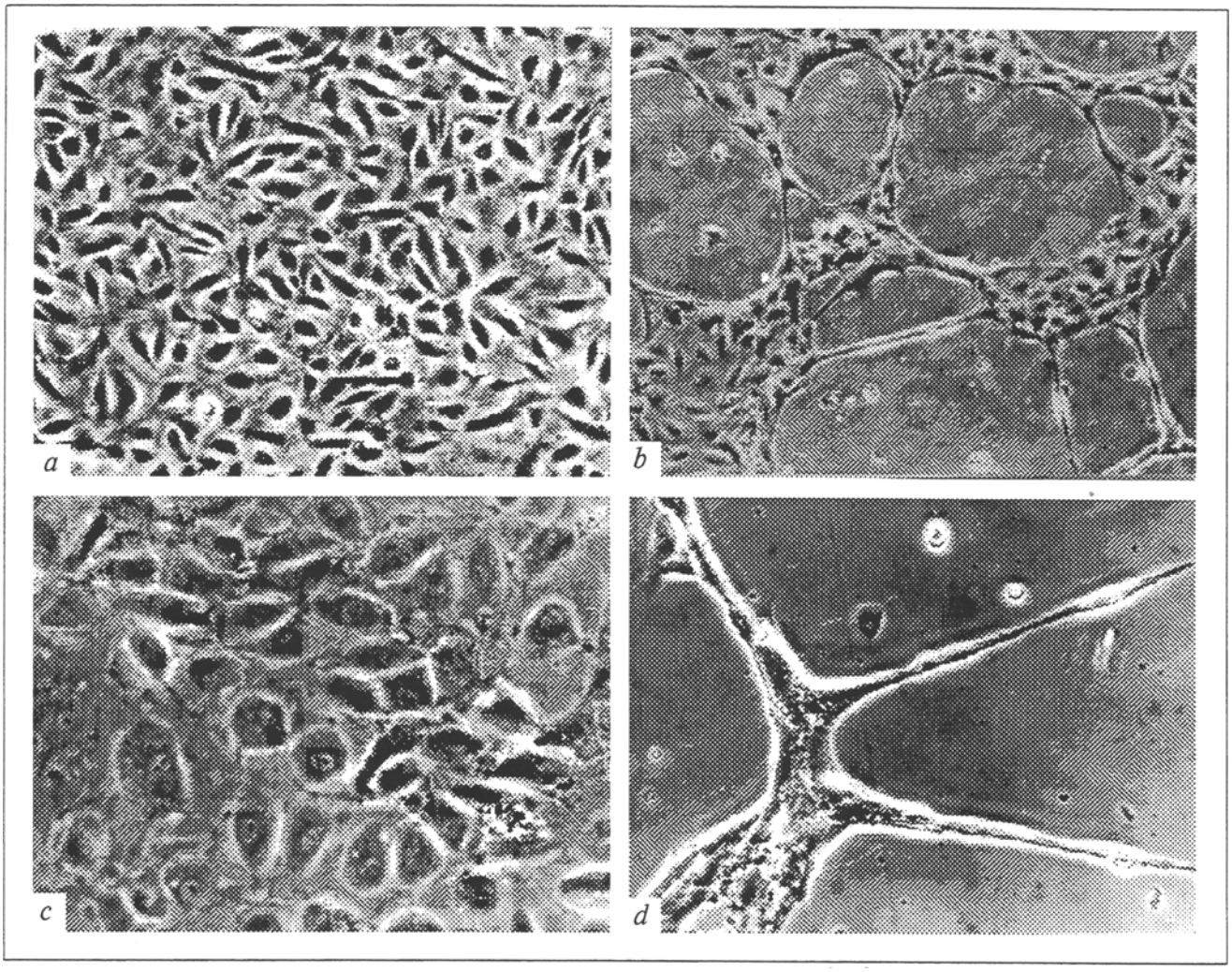

Fig. 1. *In vitro* reorganization of cultured HUV EC into capillary-like structures in the presence of SMF. Phase-contrast microscopy. *a,c*) control culture; *b,d*) exposure to SMF; *a,b*) ×60; *c,d*) ×140.

in medium 199 containing 20% inactivated human serum, 200 μg/ml endothelial growth factor (EGF) from human brain, 100 μg/ml heparin, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, and 20 mM HEPES, as described elsewhere [6]. The flasks were covered with 0.2% gelatin (Sigma) in phosphate buffer saline and air-dried. EC from bovine pulmonary artery, kindly supplied by Dr. U. Ryan [14], were cultured in DMEM supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 20 mM HEPES, 5% fetal calf serum, and 5% newborn calf serum. The cells were subcultured using 0.5% trypsin-0.02% EDTA.

For a study of the effect of SMF on attachment efficiency, the cells were placed on 35-mm Petri dishes (100,000 cells per dish), and the experimental dishes were then placed in a magnetic field with induction of 140 mT between the poles of a permanent Fe-Nd-B magnet [2]; 16-18 h later, nonattached cells were washed off with Hanks solution, and attached cells were harvested with trypsin-EDTA and counted in a hemocytometer. The effects of SMF on the growth of HUV EC were assayed as follows: EC (40,000 or 100,000) were seeded on a dish in either complete medium or medium containing only 10% or 20% human serum. Sixteen to eighteen hours later (time sufficient for cell attachment) the experimental dishes were placed in a 140-mT SMF. The media were replaced every 48 h. When the confluent state was attained in either dish, the cells were harvested with trypsin-EDTA and counted with a hemocytometer.

RESULTS

HUV EC were cultured in the presence of 10% human serum, 200 μg/ml EGF, and 100 μg/ml heparin. Under these conditions, the attachment efficiency of cells exposed to SMF virtually did not differ from that in the control cultures and was

TABLE 1. Effect of SMF on Growth of HUV EC ($M \pm m$, $n=3$)

Time in culture, days	Seeding density, 10^3 cells per dish	Content of mitogens	Number of EC per 35-mm Petri dish, $\times 10^3$	
			control	SMF exposure
6	40	10% serum 200 μg/ml EGF 100 μg/ml heparin	143±4.9	178±2.2** (124)
7	40	the same	508.3±17.3	513.0±10.9 (100)
7	40	the same	390.0±28.3	462.0±36.3 (118)
5	100	20% serum 20 μg/ml EGF 20 μg/ml heparin	585.0±6.0	588.3±4.3 (100)
5	100	20% serum	130.7±3.3	140.3±5.4 (107)
13	40	20% serum	74.7±3.8	60.7±5.9 (81)

Note. Here and in Table 2 numbers in parentheses denote the percentage of changes in comparison with the control; * – $p<0.05$, ** – $p<0.01$.

102.5±2.9% on average, while in the absence of EGF and heparin it was 94.1±5.8% of the control value. In the control cultures the efficiency of adhesion ranged from 51% to 79%.

For a study of the effect of SMF on proliferation of HUV EC the cells seeded on 35-mm Petri dishes (100,000 or 40,000 cells per dish) were cultured in a medium containing varying concentrations of serum and growth factors. In complete growth medium the cells seeded with an initial density of 40,000 cells per dish and subjected to SMF attained confluence after 6-7 days in culture. Only in one of the experimental cultures was cell propagation accelerated by 24% ($p<0.01$), while in all the others the cell count did not differ from that in the control cultures and constituted for 100-118.5%. When the concentrations of EGF and heparin were lowered to 20 μg/ml, again no effect of SMF on HUV EC propagation was observed (Table 1). As is seen from Table 1, when the cells were cultured in the presence of 20% serum, the cell count in the experimental cultures did not differ reliably from the control value and was 107.4% and 81.3% for seeding densities of 100,000 and 40,000 cells per dish, respectively.

It should be noted that a pulsed magnetic field was shown to accelerate the growth of HUV EC by 40% after the confluent monolayer was denuded [15].

In our experiments only EC of another species (bovine pulmonary artery, 13-17 passages) cultured in the medium with 10% serum had a higher proliferation rate when exposed to SMF than under control conditions. A slight (by 21.5% on average) but reliable acceleration of cell propagation was observed after 3-5 days of culturing (Table 2). A similar increase in the proliferation rate (by 20%) was observed in a culture of EC from bovine aorta exposed to a pulsed magnetic field [15].

Thus, under our experimental conditions a magnetic field with an induction of 140 mT had no positive effect on the growth of HUV EC but slightly accelerated the proliferation of EC from bovine pulmonary artery.

In subsequent experiments we studied the effect of SMF on differentiation of HUV EC. When the concentration of serum in the growth medium was lowered to 10%, HUV EC placed in SMF were found to form capillary-like structures (Fig. 1). The formation of such structures (termed *in vitro* angiogenesis) by HUV EC on a plastic substrate in a mitogen-free medium was previously shown to occur after 3-4 weeks in culture [3]. Under our experimental conditions (gelatin-precoated plastic, exposure to SMF) a similar network of capillary-like tubules was observed after 3 and 10 days in culture for seeding densities of 100,000 and 40,000 cells per dish, respectively. Without SMF no visible reorganization of the endothelial monolayer was observed over the entire observation period (10-15 days).

The *in vitro* transformation of the HUV EC monolayer into a three-demensional capillary-like

TABLE 2. Effect of SMF on Growth of EC from Bovine Pulmonary Artery ($M \pm m$, $n=3$)

Time in culture, days	Passage	Number of EC per 35-mm Petri dish, $\times 10^3$	
		control	SMF exposure
5	13	309.3±14.5	382.0±17.1* (123)
4	13	120.0±14.0	152.5±14.5 (127)
4	14	303.0±3.6	324.7±10.1 (107)
3	17	154.0±6.8	197.3±3.6** (128)

network also occurred after the omission of growth factor or substrate, or after proteolytic modification of the extracellular matrix [3,10], i.e., the differentiation of HUV EC was observed when their proliferative potential was decreased. Interestingly enough, in our experiments *in vitro* angiogenesis was induced in the presence of both serum and gelatin substrate. This effect (*in vitro* angiogenesis) was also observed under the action of a pulsed magnetic field in the presence of mitogens and fibronectin matrix. Moreover, this reorganization occurred as soon as 5-8 hours after exposure to the magnetic field [15].

Thus, SMF induces a marked acceleration of HUV EC differentiation (*in vitro* angiogenesis). The effect found by us may find practical use in medicine for the promotion of vascularization in various pathological states and in cases of impaired vascular integrity.

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