Comparative Characteristics of Platelet Lysates from Different Donors

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We studied the effect of platelet lysates from different donors on fibroblast growth in culture. In most samples (40 of 50), the growth-stimulating characteristics were greater than in 10% FCS, but every ninth sample exhibited low mitogenic activity. A weak dependence between platelet concentration and total protein content was noted, but no correlation was found between these parameters and fibroblast growth in culture.

Key Words: *platelet lysate; cell culturing; proliferation*

With increasing introduction of cell technologies into clinical practice, the problem of biological safety of materials assumes paramount importance. Animal serum routinely used for cell culturing does not comply with safety requirements, because it is a potential source of viral infections, prions, and xenogenic proteins inducing immunological reactions [11,13]. Serum-free media, growth factors, human blood components (serum, plasma, platelet-derived products) are used as substitutes of animal serum [7]. Blood platelets are a natural source of growth factors, cytokines, and adhesion molecules. Several preparations containing platelets and their derivatives can be obtained from the blood (platelet-rich plasma, platelet lysate, supernatant of activated platelets). According to published data, platelet lysate is an appropriate substitute for embryonic serum during culturing of MSC and fibroblasts, the cells most widely used in cell transplantology and tissue engineering [1,9]. However, the use of autologous blood components has certain limitations related to the amount of material and great variability of products by the content of growth factors [2,15], which can be a cause of contradictory results [12,14].

Here we studied variability of growth-stimulating properties of platelet lysates obtained from different donors.

MATERIALS AND METHODS

Platelet concentrate samples isolated from healthy donors by thrombocytopheresis were used in the experiments. Platelet lysates (PL) were prepared by triple freezing to -70°C followed by rapid thawing. The obtained PL were centrifuged at 1500*g* and 4°C for 5 min. The supernatant was used as the growth supplement to the culture medium. Total protein content in PL was measured using Bradford reagent.

Normal human skin fibroblasts at early passages were used in the experiments. Proliferative activity of fibroblasts was evaluated by growth curves. To this end, the cells were seeded in a concentration of 15,000/cm^2 in DMEM medium (HyClone) with PL to plates (3 wells per point); 10% FCS (HyClone) served as the control. Heparin (2 U, Bayer) was added for preventing medium polymerization in samples with PL. Fibroblasts were grown for 9 days with subculturing every 2 days after attaining 80% confluence. Seed-

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ing density remained the same from passage to passage. For comparison of the dynamics of cell growth under different conditions, the following parameters were calculated:

$$\text{number of population doublings } n=(\log 10(N_2)-\log10(N_1))/\log10(N_2),$$
$$\text{time of population doubling } t=T_2-T_1/n,$$

where N_2 and N_1 are cell numbers at the end and start of the experiment and T_2 and T_1 are the time of the end and start of the experiment, respectively.

The data were processed statistically using StatGraphics Centurion software. The data are presented as medians and intervals. Multiple dispersion analysis and correlation analysis were used for comparative evaluation of independent groups and detection of interrelationships, respectively. The differences were significant at $p \leq 0.05$.

RESULTS

To determine the optimum concentration of PL for fibroblast growth, different PL concentrations (from 5 to 15%) were used in series I. A tendency to activation of fibroblast proliferation with increasing PL concentration appeared by passage 3 (Fig. 1). Comparative analysis showed that wells with 10% FCS (controls) contained significantly lower number of cells by passage 4 than wells with PL in different concentrations. In experimental samples containing 7, 10, and 15% PL the results were similar. However, differences were revealed between samples containing 5 and 10% and 5 and 15% PL. Therefore, 7% PL was used in further experiments as the most optimal variant, which agrees with published data. Dose-dependent stimulating effect of platelet-derived products was previously demonstrated [3,4]. PL in concentrations of 5-10% are usually used for mesenchymal cell culture [6,10].

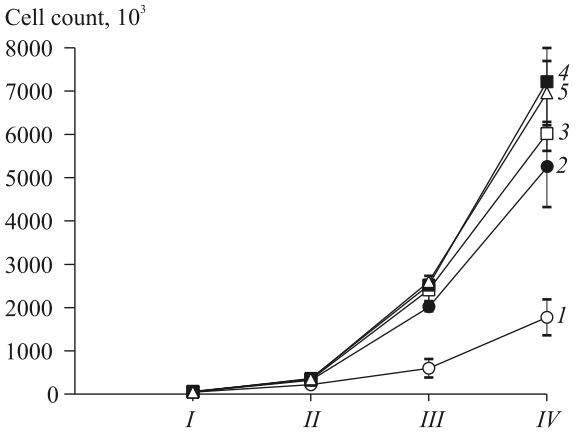

Fig. 1. Dynamics of growth of normal human skin fibroblasts during culturing under standard conditions with 10% FCS (*1*), 5% PL (*2*), 7% PL (*3*), 10% PL (*4*), and 15% PL (*5*). Abscissa: passage number.

A total of 50 PL samples were analyzed. For each sample, the number (n) and time (t) of population doubling were determined (means and standard deviations) and the groups were compared by these parameters. All samples were divided into groups according to the obtained results and the minimum and maximum n and t values were specified for each group (min-max; Table 1). Forty of 50 samples exhibited significantly higher growth-stimulating activity compared to serum, the number of doublings varies from 5.8 to 10.2, 4 samples did not differ from serum, 4 inhibited fibroblast proliferation (doubling time <5), and 2 samples induced death of the culture by passage 4.

For evaluation of the effect of these differences on the increase in total cell mass, the ratio of the cell count by passage 4 to that in the control (cell growth in the presence of ETC; Fig. 2). The majority of PL samples caused a 2-4-fold increase in cell mass increment compared to the control, while some PL samples provide a 12-16 increase in cell mass increment.

Possible correlation between platelet concentration, total protein content in PL, and growth-stimu-

TABLE 1. PL Groups by the Main Parameters

PL group	Number of samples	Concentration		n (min-max)	t (min-max)
		protein, mg/ml	platelets, 10^{11}/ml		
Control (FCS)	-	35	-	5.3	40.8
n above control ($p \leq 0.05$)	40	42.0-61.5	3.5-6.2	5.8-10.2	21.2-37.2
n below control ($p \leq 0.05$)	4	49.5-59.6	4.5-6.2	2.8-5.1	42.4-77.1
Did not differ from control	4	49.5-59.6	4.2-4.4	5.2-5.5	39.3-41.5
Caused death	2	47.5-67.6	4.5	0	0

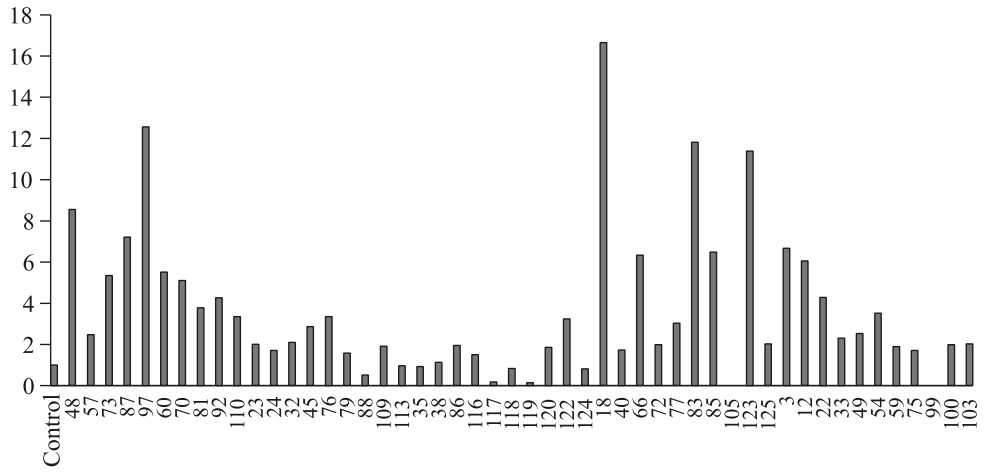

Fig. 2. Changes in total increment of fibroblast number by day 9 in culture. Abscissa: variants of culturing; ordinate: coefficient K reflecting the ratio of cell count increment during culturing with different PL samples to that in the control (taken as 1).

lating characteristics was studied using correlation analysis (Table 2). There is a weak correlation between platelet concentration in the sample and total protein content, which reflects low variability of these parameters. No correlations between these parameters and cell growth in the culture were found.

The proliferation-stimulating activity of PL is determined by the presence of growth factors, in particular PDGF and TGF-β. The concentrations of growth factors can vary from sample to sample [2,15]. It can be hypothesized that in PL with growth-stimulating activity lower than that of FCS the content of referent growth factors is insufficient. There are data that not only growth factors, but also adhesion molecules can be the determinative causes; for instance, the antiproliferative effect of platelet concentrate can be determined by the presence of thrombospondin [5]. The use of autologous platelet concentrate as a serum substitute can be low effective, because the incidence of PL samples with low growth-stimulating characteristics in rather high (every ninth sample). There are data that the stimulating effect of platelet-rich plasma manifests at platelet concentration $>10^6/\mu l$ [8], but such PL characteristics as total protein content and platelet count cannot serve as measures of quality. For leveling the individual differences between the samples, it is advisable to pool PL from at least 10 donors. For researchers working in the field of cell technologies, the priority problems are the development of standardized protocols of obtaining FCS substitutes from platelet products and the choice of parameters for evaluation of their quality for obtaining guaranted proliferative response.

TABLE 2. Correlations between PL parameters

Parameter	Platelet count	Protein concentration
Doubling time	r=0.106 (p=0.4748)	r=-0.055 (p=0.710)
Number of doublings	r=-0.067 (p=0.6526)	r=-0.047 (p=0.7508)
Platelet count		r=0.3032 (p=0.0362)

Note. r: coefficient of correlation.

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