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Original Article

Effect of Different Plasma Concentrations Human Plasma and Goat Plasma on Human Blood Cell Growth *in Vitro*

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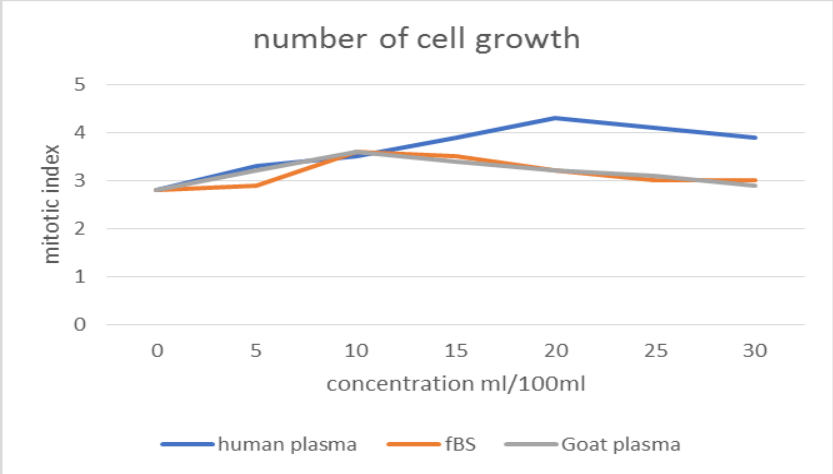
ABSTRACT

Cultivated media for the growth of human blood cells are many, but the use of natural and less expensive materials is the most important to obtain the suitable growth for the cultivation of human lymphocytes. It needs a lot of materials that help in its development. Here, plasma is a very important growth factor for the growth of cells, which through its growth is useful in studying many natural and abnormal processes and interactions that occur in cells or studying the effect of many chemicals on cells through the examination of cell division so that it does not affect the nature of their chromosomes, and the results showed that the best concentration of chromosomes. Human plasma suitable for the growth of human blood cells is 20% and 10% plasma concentration which gave the highest cell division rate that could be obtained. There are no significant differences between plasma concerning the cell division index. As for the study of the chromosomal body, this indicator is important and is widely used in cytogenetic studies.

GRAPHICAL ABSTRACT



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Introduction

Lymphocytes have been used extensively, in several clinical trials due to their immunomodulatory and regenerative properties. Like the levels at which these cells are located the medium used for cell culture is a major compliance factor [1, 2]. Although effective, the use of fetal bovine serum (FBS), which is currently the standard supplement for cell expansion, or other animal-derived ingredients is strongly discouraged by regulators because of the risk of transmission of foreign infectious agents and immunization [3, 4]. Therefore, the scientific community suggests the alternatives to FBS. Chemically determined supplement successfully used to produce recombinant therapeutic proteins. It is not effective in supporting lymphocyte growth. Its use indicates the components derived from human blood such as human plasma (autologous or allogeneic complex) and they are available so far [5]. The potential risks of these products are reduced because the components of human blood have been in clinical use for years. Derived from healthy blood donors and tested according to the blood bank criteria for infectious and immunological agents [5]. Among the components derived from blood, human AB plasma (AB) can be considered as a promising alternative to FBS due to its availability and potential for self-use [6]. Human AB serum is used to culture human cell lines, including human stem cell lines. Human AB serum is prepared from naturally clotted whole blood. Human serum from clotted whole blood is most often used since it contains more growth factors. Various studies mentioned the successful use of this supplement for various cell types; including CSCs are located in niches, anatomically distinct regions within tumor microenvironment. These niches maintain the essential properties of CSCs, maintain phenotypic flexibility, protect against the immune system, and promote metastatic potential [7]. Goat plasma was extracted from goats from animal species and are few preliminary reports on goat and tissue culture [8, 9]. However, none of those studies have looked at different sources for the AB production. This study aims to evaluate human and goat plasma concentrations to support lymphocyte growth.

Materials and Methods

Human plasma, type AB, taken from a blood bank, and goat plasma collected from goat breeding fields were used, and then placed in a water bath at 56 °C for 1 hour. Next, it was divided into special tubes and kept at -20 °C until use for comparison with serum. Bovine embryo is commonly used in laboratory culture media. Various concentrations of human plasma were used (0-30 mL/100 mL medium) medium type RPMI1640. Approximately 0.5 mL heparinized whole blood was inoculated into a glass or plastic tube with 10 mL of medium, and incubated the culture at 37 °C for 72 hours. 0.5 ug/mL of colcemid solution was added to each culture tube. The culture was incubated for an additional 15-30 min. The culture was transferred to a centrifuge tube and spun at 500 g for 5 min. The supernatant was removed and the cells were re-suspended in 5-10 mL of hypotonic 0.075M KCl, and then incubated at 37° C for 10-12 min, spun at 500 g for 5 min, and the supernatant was removed. Thereafter, the cellular sediment was stirred and 5-10 mL dropwise of the fresh, chilled stabilizer of 1-part acetic acid was added to 3 parts methanol. Then, it was left at 4 °C for 10 minutes. The spin steps were repeated at 500 g for 5 min. The cell pellet was re-suspended in a small volume of 0.5-1 mL of new fixative; it was dropped onto a clean slide, and allowed to air dry. At this point, the lotion could be tinted with Giemsa [10, 11]. Giemsa ligation had become the most widely used technique, and the most common method for obtaining this staining is to treat slices with Trypsin-EDTA 10X.

Statistical analysis

The data have been analyzed by the IBM SPSS statistics program for windows. All of the data in this study expressed likewise mean ± standard deviation (SD), and the data have been analyzed by using T-test independent samples. Differences have been examined to be significant for values of p-value < 0.05 and 0.01.

Results and Discussion

Blood samples were taken from healthy subjects. A human blood sample was cultured in three RPMI1640 culture mediums with added concentrations of AB plasma, another medium to which was added goat plasma, and the last medium to which fetal bovine serum was added and included concentrations (0, 5, 10, 15, 20, 25, and 30% Ml). After harvesting the cells, 1000 divided cells were counted. To measure the mitotic index (MI), the frequency of metaphase nuclei, as in Table 1 and Figures 1 and 2, the mitotic index assay was used to assess the genotoxicity of physical and chemical substances on the mitotic human lymphocyte cell. The indicator is the percentage of nuclei in the nuclei of the mitotic stage in a thousand cells, as in the following equation.

$$\%MI \frac{\text{No. of Dividing cell}}{\text{No. of dividing and nondividing cells}} \times 100$$

It was found that increasing the concentration increases cell division and growth to a concentration of 20%. The dividing cells begin to decrease in the mitotic index in the culture media that contains human plasma, while in the media containing the concentrations of goat plasma and fetal bovine serum, the best concentration was 10%. There are no significant differences between plasma concerning the cell division index. As for the study of chromosomal body, this indicator is important and widely used in cytogenetic studies. Evidently obtaining cells that enter the tropics phase at 20% plasma concentration is also better than the remaining concentrations and reaches 8.7% compared with the remaining concentrations, as presented in Table 2.

Table 1: Mitotic index in different concentrations of human plasma AB

Type of growth Factors	Percentage of plasma or serum concentration						
	MI. 0%* (mean +S. D)	MI.5%* (mean +S. D)	MI.10%* (mean +S. D)	MI.15%* (mean +S. D)	MI. 20%* (mean +S. D)	MI.25%* (mean +S. D)	MI.30%* (mean +S. D)
Human plasma	2.8 + 0.10	3.3 + 0.21	3.5 + 0.33	3.9 + 0.41	٤.30+ 0.53	4.1 + 0.44	3.9 + 0.41
FBS***	2.8 + 0.09	2.9 + 0.12	3.6 + 0.05	3.5 + 0.11	3.22+ 0.21	3.0 + 0.12	3.0 + 0.05
Goat plasma	2.8+ 0.10	3.2 + 0.22	3.6 + 0.10	3.4 + 0.05	3.21 +0.11	3.1 + 0.11	2.9 + 0.10
P-value	P >0.05**						

**Percentage of plasma concentration, standard deviation S.D, and Mitotic index MI, ** non-significant, ***fetal bovine serum.*

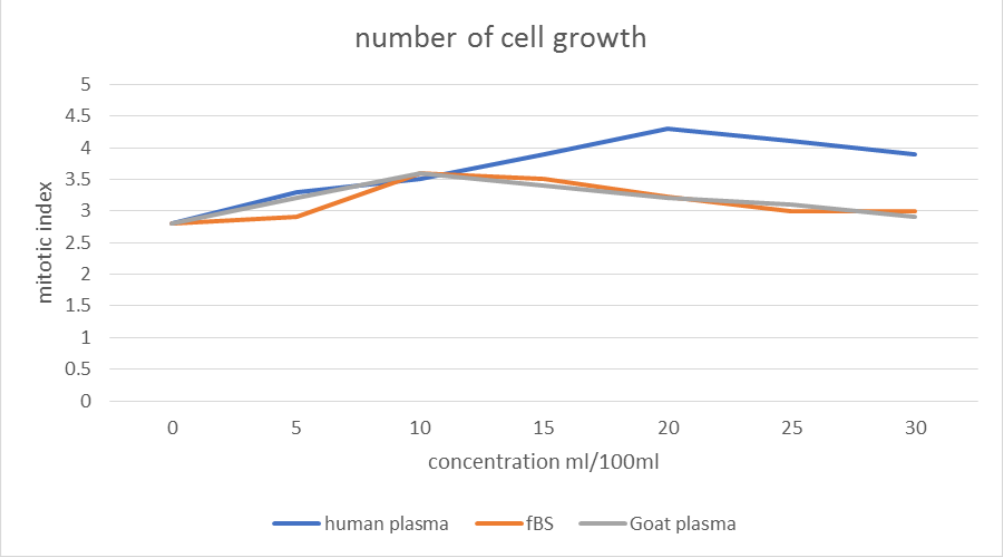

Figure 1: Growth of cells with different concentrations of plasma in a culture medium

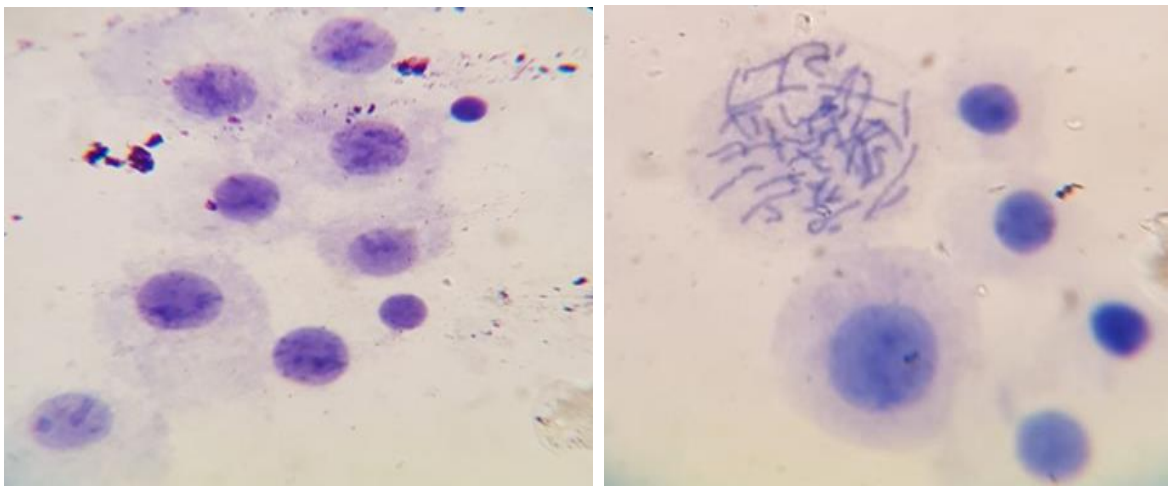

Figure 2: Growth of cells at concentrations of 20% of the human plasma in the culture medium (400X)

Table 2: Percentage of metaphase with different concentrations of AB plasma or fetal calf serum in culture media

Type of growth factors	Number of metaphase and concentration of human plasma or fetal calf serum						
	No. Metaphase% 0%*	No. Metaphase% 5%	No. Metaphase% 10%	No. Metaphase% 15%	No. Metaphase% 20%	No. Metaphase% 25%	No. Metaphase% 30%
Human plasma	2.01	3.31	5.43	6.33	8.70	7.66	3.40
FBS***	2.00	3.30	5.41	6.32	8.69	7.60	3.38
Goat plasma	2.01	3.20	5.42	6.23	8.56	7.55	3.33
P-value	P >0.01**						

**Number of metaphase and concentration of human plasma, , **non-significant, ***fetal bovine serum.*

Human plasma was used in the culture of lymphocytes as if they were living in normal conditions, and its addition to the medium commonly used in the culture of lymphocytes is RPMI 1640, which contains glucose, amino acids, vitamins, and salts in concentrations that are not found in large part. Those concentrations are reflected from human plasma, whereas basal media are often supplemented with heat-inactivated fetal bovine serum (IFS), which contributes nonspecifically and often as growth factors and hormones necessary for cell proliferation, a common treatment for FBS is heat inactivation. In this case, the FBS is heated in a water bath at 56 °C for 30 minutes with occasional shaking. Its purpose is to inactivate any component of the complement system present in FBS and potential unknown inhibitors of cell proliferation [12]. Cells grown in media that better reflect the metabolite range of human plasma are largely unexplored, and conventional human plasma (SCP) serum contains platelet-derived growth factor (PDGF). This growth factor is a potent inhibitor of adipogenesis and is also known as the most important inducer of proliferation in human serum. Through previous research, human AB serum is used in tissue engineering, organ transplantation, and cell therapy applications to expand mesenchymal stem cells (MSCs) from adipose tissue or mesenchymal stromal cells from human bone marrow to generate and transplant standard limbal epithelial stem cell grafts for the *ex vivo* expansion of natural killer cells from peripheral blood amid hematopoietic stem cell expansion upgrading the technology of human islet culture before transplantation. To begin to its address, different concentrations of human plasma were used and compared with naturally available goat plasma and fetal bovine serum, which is frequently used in the laboratory. Human plasma was taken from healthy adult humans (HPLM) consistent with it [13-15]. It was also compared with a medium devoid of any type of plasma or

serum and found that cells growth required careful conditioning of growth factors to support the perforations of different cell types. Among the concentrations of the plasma types used in the research, it was found that the ideal concentration for culture is 20% human plasma and 10% goat plasma and bovine serum so that it does not affect the cell's growth and nature of chromosomes, as presented in Tables 1 and 2 and Figures 1 and 2 and this result is consistent with other studies. Thus, HPLM will be of great benefit, to the proliferation of a wide range of cells.

Conclusion

According to the results, it was concluded that the use of AB plasma in the culture medium RPMI1640 was possible through studying the rate of mitosis and obtaining recombinant cells at an equal or even better rate than fetal cow's serum, as a xeno-free product may facilitate the fulfillment of safety and efficacy requirements for clinical studies.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

There are no conflicts of interest in this study.

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