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Assessment of the functions of cultured lymphocytes when exposed to drugs used in cosmetology

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ABSTRACT

The aim of the study was to determine the number of lymphocytes, intracellular cytokines produced by lymphocytes, and the cell cycle of lymphocytes isolated from the blood of patients when exposed to various drugs, as well as to assess the functions of cultured lymphocytes when exposed to drugs *in vivo* and *in vitro*.

Materials and methods. The study involved lymphocytes isolated from the blood of healthy women under various conditions. At the first stage of the study, T-lymphocytes were isolated from the blood of patients before exposure to the drug. The absolute and relative lymphocyte count, the number of intracellular cytokines, and the cell cycle were determined.

At the second stage, the drugs were added to the nutrient medium, where lymphocytes isolated from the blood of patients who did not receive systemic drugs were cultured. The placental extract preparation was added to the lymphocytes isolated from the first group of patients, while the hyaluronic acid preparation was added to the lymphocytes isolated from the second group of patients.

At the third stage, the lymphocytes isolated from the blood of patients after systemic exposure to the placental extract preparation or hyaluronic acid preparation were isolated and cultured, after which the same lymphocyte parameters were determined.

Results. The number of T-lymphocytes increased with the systemic use of the placental extract and hyaluronic acid preparations and practically did not change compared with the baseline data, when these drugs were added to the nutrient medium. Placental extract and hyaluronic acid had a positive effect on the mitotic activity of cells; it is worth noting that the effect of placental extract was greater than that of hyaluronic acid. Both drugs did not have a negative effect on apoptosis of T-lymphocytes. Under the effect of placental extract, lymphocytes secreted more interleukins, which contributed to proliferation of keratinocytes.

Conclusion. The placental extract and hyaluronic acid preparations have a stimulating effect on keratinocytes. The placental extract preparation has a stimulating effect on T-lymphocytes after systemic exposure of the body to it.

Keywords: lymphocyte culture, intracellular cytokines, placental extract, hyaluronic acid

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Оценка функций культивированных лимфоцитов при воздействии препаратов, использующихся в косметологической практике

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РЕЗЮМЕ

Цель – определить количество лимфоцитов, внутриклеточных лимфоцитарных цитокинов, клеточный цикл лимфоцитов, выделенных из крови пациентов при воздействии различных препаратов, оценить функции культивированных лимфоцитов при воздействии препаратов *in vivo* и *in vitro*.

Материалы и методы. Исследованию подвергались лимфоциты, выделенные из крови здоровых женщин при различных условиях. На первом этапе исследования выделялись Т-лимфоциты из крови пациентов до воздействия препарата. Определялись: абсолютное и относительное количество лимфоцитов, внутриклеточные цитокины, клеточный цикл.

На втором этапе в питательную среду, где культивировались лимфоциты, выделенные из крови пациентов, которые не получали системно препараты, добавлялись препараты. К лимфоцитам первой группы пациентов в питательную среду добавлялся препарат экстракта плаценты, к лимфоцитам второй группы пациентов – препарат гиалуроновой кислоты.

На третьем этапе выделялись и культивировались лимфоциты, которые были выделены из крови пациентов после системного воздействия на организм препарата экстракта плаценты или препарата гиалуроновой кислоты, после чего определялись те же показатели лимфоцитов.

Результаты. Количество Т-лимфоцитов увеличивалось при системном использовании препаратов экстракта плаценты и гиалуроновой кислоты и практически не менялось по сравнению с исходными данными при добавлении этих препаратов в питательную среду. Экстракт плаценты и гиалуроновая кислота положительно влияют на митотическую активность клеток, экстракт плаценты в большей степени, чем гиалуроновая кислота. Оба препарата не оказывают негативного влияния на процессы апоптоза Т-лимфоцитов. При действии экстракта плаценты лимфоциты выделяют больше интерлейкинов, которые способствуют пролиферации кератиноцитов.

Заключение. Препараты экстракта плаценты и гиалуроновой кислоты оказывают стимулирующее действие на кератиноциты. Препарат экстракта плаценты оказывают стимулирующее действие на Т-лимфоциты при системном воздействии на организм.

Ключевые слова: культивация лимфоцитов, внутриклеточные цитокины, экстракт плаценты, гиалуроновая кислота

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

The use of drugs based on human placental extract is one of the actively studied and developing areas in aesthetic medicine and many other branches of medical science. The human placental extract is an active complex that encompasses amino acids, enzymes, including those for antioxidant defense, vitamins, minerals, growth factors, immunotropic substances, etc. There are published data on the stimulating effect of human placental extract preparations on regeneration of various tissues [1, 2], as well as on the regulating effect of the placental extract on inflammatory processes [3].

In cosmetology, hyaluronic acid preparations are widely used to improve the quality of the skin. Hyaluronic acid promotes water retention in the dermis, has a stimulating effect on fibroblasts, which leads to an increase in the number of collagen fibers in the dermis, and neutralizes the action of proinflammatory interleukins in the skin, which contribute to skin aging [4–7].

The literature presents an increasing amount of data on changes in the skin after exposure to drugs that promote rejuvenation, but there is practically no information about the immune response to administration of such drugs.

The aim of the study was to determine the number of lymphocytes, intracellular cytokines produced by lymphocytes, and the cell cycle of lymphocytes isolated from the blood of patients when exposed to various drugs, as well as to assess the functions of cultured lymphocytes when exposed to drugs *in vivo* and *in vitro*.

MATERIALS AND METHODS

The study involved lymphocytes isolated and cultured from the blood of healthy women under various conditions. At the first stage of the study, T-lymphocytes were isolated from the blood of patients before they were injected with placental extract and hyaluronic acid preparations. These lymphocytes were cultured in a nutrient medium, after which the absolute and relative lymphocyte count, the number of lymphocytes undergoing apoptosis and intracellular cytokines produced by lymphocytes, and the cell cycle were determined.

At the second stage, drugs were added to the nutrient medium where lymphocytes isolated from the blood of patients who did not receive the drugs systemically were cultured. A placental extract preparation was added to the nutrient medium with lymphocytes isolated from the first group of patients, and a hyaluronic acid preparation was added to the lymphocytes isolated from the second group of patients (exposure to the drugs *in vitro*). After a certain incubation period, the same parameters were determined as at the first stage of the study.

The third stage involved isolating and culturing lymphocytes from the blood of patients after systemic exposure of the body to the placental extract or hyaluronic acid preparation (exposure to the drugs *in vivo*), after which the same parameters of lymphocytes were measured.

The data obtained were subject to statistical processing. According to the Kolmogorov – Smirnov test, the sampling distribution was incorrect; therefore, nonparametric methods of statistical processing of the obtained data were used. The *Mo* mode, median, and interquartile range *Me* (*Q*25/ *Q*75) were determined. The calculation was carried out using the IBM SPSS Statistics 2 software packages.

RESULTS

At the first stage, the initial mean absolute cell count of T-lymphocytes isolated from the blood of patients who were not exposed to any drugs was 15,448 cells, and the relative value was 97.2%. After exposure to the placental extract preparation *in vitro*, these parameters practically did not change and amounted to 15,749 cells and 97.0%, respectively. When exposed to the placental extract preparation *in vivo*, the absolute cell count of T-lymphocytes increased significantly and amounted to 19,402.0 cells in the nutrient medium.

When exposed to hyaluronic acid, the absolute and relative cell count of T-lymphocytes decreased both *in vivo* and *in vitro*. *In vitro,* the number of T-lymphocytes was 14,888 cells, *in vivo* – 12,349 cells, which indicated a more pronounced decrease in the lymphocyte count with systemic exposure of the body to the hyaluronic acid preparation. The same tendency was observed in the change in the relative lymphocyte count: 95.3 and 95.1% in *in vitro* and *in vivo* exposure, respectively.

Evaluating the number of proliferating cells that were in the G2 stage and M stage of the cell cycle (the synthetic and mitotic phases, indicating active cell proliferation), we found an increase in this parameter in patients of both groups who received the placental extract and hyaluronic acid preparations. The increase occurred when the individuals were exposed to drugs both *in vitro* and *in vivo*. So, when exposed to the placental extract, the absolute number of proliferating cells increased from 143 to 276 *in vitro* and to 887 *in vivo*, and when exposed to hyaluronic acid, the cell count rose to 396 and 194 cells, respectively. If we compare the degree of increase when exposed to different drugs, we can note a more pronounced degree of cell growth during exposure to the placental extract, which is probably due to the immunomodulating properties of this preparation.

The relative value of proliferating cells also increased, and the tendency was the same as with the increase in the absolute count of proliferating T-lymphocytes. The preparations had practically no effect on the number of apoptotic cells. The absolute and relative lymphocyte count in apoptosis practically did not change when exposed to both preparations either *in vitro*, or *in vivo*, which may indicate that the placental extract and hyaluronic acid preparations did not have a negative effect on the body.

The DNA index, that is the ratio of cells whose cell cycle was in the G1 and G2 phases, practically did not change when exposed to different drugs. The results are presented in Table 1.

Table 1

The number of cultured lymphocytes and lymphocyte cell cycle parameters before and after exposure to drugs *in vitro* and *in vivo*

Parameter	Statistical parameters	Lymphocyte culture				
		without exposure, *n* = 12	with the addition of placental extract *in vitro*, *n* = 12	after systemic exposure to placental extract *in vivo*, *n* = 12	with the addition of hyaluronic acid *in vitro*, *n* = 12	after systemic exposure to hyaluronic acid *in vivo, n* = 12
Absolute count						
Normal cells (G0/G1)	Mo	2,480	15,874	8,708	11,153	14,333
	Me (*Q*25/*Q*75)	15,448 (1,476.0/1,570.0)	15,749 (13,704.0/17,169.0)	19,402 (12,184.0/46,790.0)**	14,888 (9,321/16,291)	12,349 (6,765/14,223) **
Proliferating cells (G2, M)	Mo	28	31	18	6	70
	Me (*Q*25/*Q*75)	143 (26.25/443.7)	276 (83.7/415.5)*	887 (242.0/1,556.0)**	396 (119.7/586.0)**	194 (80.2/432.7)
Apoptotic cells	Mo	10.0	4.0	0.8	42.0	4.0
	Me (*Q*25/*Q*75)	37 (3.75/55.7)	31.5 (10/53)	42 (4.5/81.5)	19.5 (4.5/42.5)	37.5 (14/66)
Relative count, %						
Normal cells (G0/G1)	Mo	95.2	97.8	82.7	62.3	82.1
	Me (*Q*25/*Q*75)	97.2 (95.2/98.7)	97 (93.6/97.8)	96.4 (85.6/99.2)	95.3 (90.7/97.3)	95.1 (89.0/97.2)
Proliferating cells (G2, M)	Mo	0.1	0.2	0.1	0.2	0.51
	Me (*Q*25/*Q*75)	0.15 (0.1/0.91)	0.58 (0.17/1.62) **	1.4 (0.42/4.35) **	0.64 (0.31/1.22) **	0.87 (0.62/1.32) **
Apoptotic cells	Mo	0.1	0.1	0.3	0.3	0.4
	Me (*Q*25/*Q*75)	0.3 (0.1/2.3)	0.4 (0.1/1.67)	0.3 (0.15/1.45)	0.35 (0.3/0.55)	0.55 (0.32/0.9)
DNA index (G1/G2)	Mo	1.93	1.96	1.67	1.96	0.97
	Me (*Q*25/*Q*75)	1.93 (1.84/2.08)	1.97 (1.91/12.11)	2.01 (1.9/6.52)	1.97 (1.96/2)	1.96 (1.85/2)

** $p < 0.01$, *$p < 0.05$ – significance of differences between the parameters before and after exposure to drugs *in vitro* and *in vivo*.

When assessing the level of intracellular cytokines that cultured T-lymphocytes secreted into the nutrient medium, we found a decrease in the content of interleukin (IL)-1b produced by the CD4+ subpopulation. A decrease in the level of this cytokine occurred when both the placental extract and hyaluronic acid were added to the nutrient medium. The lymphocyte subpopulation with the CD8+ phenotype produced this cytokine more with the addition of placental extract than with the addition of hyaluronic acid. Interleukin IL-17A increased in the nutrient medium where lymphocytes with the CD4+ phenotype were cultured with the addition of placental extract and decreased when hyaluronic acid was added. The content of interleukin IL-17A produced by lymphocytes with the CD8+ phenotype increased with the addition of placental extract and hyaluronic acid. The data are presented in Table 2.

Table 2

The number of intracellular cytokines before exposure to drugs and after adding drugs to the nutrient medium

Parameter	Statistical parameter	Spontaneous cytokine production		
		by lymphocytes, *n* = 12	with the addition of placental extract to the medium, *n* = 12	with the addition of hyaluronic acid to the medium, *n* = 12
CD4+(IL1b)	Mo	4.0	25.0	3.0
	Me (*Q*25/*Q*75)	12.0 (4.0/61.25)	8.0 (3.75/26.75)**	8.5 (4.5/56.0)**
CD8+(IL1b)	Mo	7.0	17.0	7.0
	Me (*Q*25/*Q*75)	8.5 (2.0/20.25)	10.5 (6.25/17.0) **	7.0 (6.0/7.5)
CD4+(IL-17A)	Mo	1.0	2.0	4.0
	Me (*Q*25/*Q*75)	6.0 (1.0/19.0)	9.0 (6.5/24.0)**	4.0 (3.0/4.0)**
CD8+(IL-17A)	Mo	4.0	8.0	9.0
	Me (*Q*25/*Q*75)	6.0 (4.0/10.0)	8.5 (7.75/18.0)*	9.0 (9.0/12.0)*

**$p < 0.01$, *$p < 0.05$ – significance of differences between the parameters before and after exposure to drugs *in vitro* and *in vivo*.

DISCUSSION

The number of T-lymphocytes upon exposure to placental extract increased *in vivo* and practically did not change *in vitro*. This may indicate that the described immunomodulating effect of the placental extract will be realized under the condition of a complex effect on the body immune system, where all the links of the immune system are connected with each other and are in close interaction.

On the other hand, the number of lymphocytes upon exposure to hyaluronic acid decreased *in vivo* and did not change *in vitro*. Apparently, this is due to the need to eliminate a foreign substance, which is hyaluronic acid, administered via an intradermal injection, and lymphocytes are consumed in this process.

Both placental extract and hyaluronic acid stimulate mitosis and cell proliferation. With the systemic effect of the placental extract, this process is more active in comparison with the systemic effect of hyaluronic acid, which may be due to a more pronounced positive effect of the placental extract on the immune system and the body as a whole. These drugs have no effect on apoptosis of lymphocytes, which may indicate safety of these drugs for the immune system.

Estimating the content of interleukins that have a stimulating effect on proliferation of keratinocytes, it can be said that the total level of IL-1b and IL-17A increases to a greater extent under the effect of placental extract than hyaluronic acid. On this basis, we can speculate about greater effectiveness of the placental extract in relation to skin cell renewal, although hyaluronic acid has a similar effect.

CONCLUSION

Placental extract has a stimulating effect on lymphocytes upon systemic exposure of the body to it. The results of the study allow us to speculate about the stimulating effect of placental extract and hyaluronic acid not only on the immune system, but also on the quality of the skin.

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