



## RESEARCH ARTICLE

Open Access

## Development of a New Non-Invasive Approach to Treat of Infantile Hemangioma with Growth-Inhibiting Endogenous Factors (Non-Invasive Approach to Treat of Infantile Hemangioma with Endogenous Factors)

Diana Dzidziguri<sup>1\*</sup>, Elza Markarashvili<sup>1</sup>, Irina Modebadze<sup>1</sup>, Levan Rusishvili<sup>1</sup>, Nino Kharashvili<sup>1</sup>, Tornike Leverashvili<sup>1</sup>, Zurab Vadachkoria<sup>2</sup>, Giorgi Ghibradze<sup>2</sup>, Lia Dzidziguri<sup>2</sup> and Manana Berulava<sup>3</sup>

<sup>1</sup>Chavchavadze av. N3, 01028, Tbilisi, Ivane Javakhishvili Tbilisi State University, Georgia

<sup>2</sup>Vazha-Pshavela Ave. 33, 0186 Tbilisi, Tbilisi State Medical University, Georgia

<sup>3</sup>61 Politkovskaya street, 0186, Sokhumi State University, Georgia

### ABSTRACT

**Background:** Infant hemangioma is a benign vascular tumor, the main reason for the development of which is genetic disorders occurring in embryogenesis. It should be noted that all methods of treating the disease, both general and local, have potential negative risks and side effects. The identification of endogenous regulatory factors and researching the possibilities of using them for treatment is relevant. There is the thermostable proteins complex (TPC) which has been identified in the cells of phylogenetically distant almost all organisms, which has the ability to decrease the mitotic activity of cells by inhibition of transcription. In the experimental model of hemangioma, it has been established that inhibition of capillary growth can only be achieved by injection. The aim of the work was to develop a new, less invasive method of hemangioma treatment with endogenous factors.

**Research Object and Material:** Adolescent white rats and adult hens; the liver and peripheral blood of adolescent rats and the liver of the adult hens.

**Methods:** Isolation of TPC from hens' liver; immobilization of TPC in the polyvinyl alcohol functional polymer; determination of colchicine mitotic index; immunohistochemical analysis.

**Results:** The polyvinyl alcohol functional polymer does not effect on the peripheral blood leukocytes and on morphology of the adolescent rats' liver. The hen's liver TPC injected into the functional polymer retains the ability to suppress the proliferative activity of the homologous tissue of adolescent rats. At the same time, based on immunohistochemical analysis amount of ki67 positive cells is increased. Similar result was obtained on the mitotic activity of the adolescent rats' liver when the exogenous calmodulin introduced by polymer film.

#### Conclusion:

1. The polyvinyl alcohol functional polymer does not have a negative effect on the peripheral blood and the liver histoarchitectonics of the adolescent white rats.
2. The protein complex immobilized into a functional polymer and its active component, calmodulin, retains the ability to suppress the proliferative activity of the adolescent rat liver.
3. There is a possibility of therapeutic use of polyvinyl alcohol functional polymer film and exogenous calmodulin immobilized into it.

**Keywords:** Infantile Hemangioma, Endogenous Factors, Mitotic Activity, Calmodulin

### Introduction

Hemangioma belongs to the most common vascular benign tumors of newborns, which includes 50% of soft tissue tumors. That is

why, due to special attention to this disease, several approaches to the treatment of hemangiomas have been developed. At the same time, it is known that modern medicine cannot always provide a quick and easy cure for vascular anomalies and tumors with diverse clinical manifestations. For example, radiation therapy and corticosteroid treatment of hemangiomas are effective, but

### ARTICLE HISTORY

Received May 06, 2024  
Accepted May 14, 2024  
Published May 21, 2024

### KEYWORDS

Infantile Hemangioma,  
Endogenous Factors, Mitotic  
Activity, Calmodulin

**Contact:** Diana Dzidziguri, Chavchavadze av. N3, 01028, Tbilisi, Ivane Javakhishvili Tbilisi State University, Georgia.

have well-known risks (neuropathy, growth disorders, anorexia, adrenal insufficiency). For example, sclero destruction with 70% ethyl alcohol has good results. However, it should be noted that its injection is very painful and requires general anesthesia. Due to the high risk of intraoperative bleeding, special sensitivity to blood loss at children's age, localization mainly in the maxillofacial area, the formation of post-operative scars and other reasons, excision of hemangiomas is used in only 1% of cases [1,2]. The above-mentioned circumstances lead to a growing interest in the search for new methods of non-operative treatment of hemangioma.

From this point of view, it is relevant to identify the endogenous factors suppressing cell proliferation and evaluate the possibilities of using them for the treatment of hemangiomas. In the cells of almost all phylogenetically distant organisms, from bacteria to humans (including infantile capillary hemangioma cells), we have identified a thermostable protein complex (TPC - protein components in the 11-200 kDa range) and it has been established that the low-molecular component of the complex (calmodulin) has the ability to inhibit the mitotic activity of cells by inhibiting transcription [3-5].

Therefore, we considered it interesting to study in detail the activity of the thermostable protein complex suppressing proliferation in hemangioma cells and to develop a new, non-invasive method for their future use in the treatment of hemangiomas.

The aim of the work was to study the effect of hen's liver protein complex immobilized in a polyvinyl alcohol functional polymer on the proliferative activity of liver cells of adolescent rats.

### Materials and Methods

White adolescent non-linear rats (7 days old) and adult hens (two months old) were selected as a research objects. The study materials were the liver and peripheral blood of adolescent rats and the liver of the hens. TPC from the hen's liver was obtained by Bullow's alcohol extraction method [6].

Immobilization of TPC and calmoduline in the polyvinyl alcohol functional polymer: TPC and exogenous calmodulin 0.2-0.1 mg/ml are added to the aqueous solution of the polyvinyl alcohol polymer. After mixing well, it is poured into the Teflon frame and after 48 hours, 50  $\mu$ m thick and 1.5 cm diameter films are obtained, in which the active substance is immobilized [7,8].

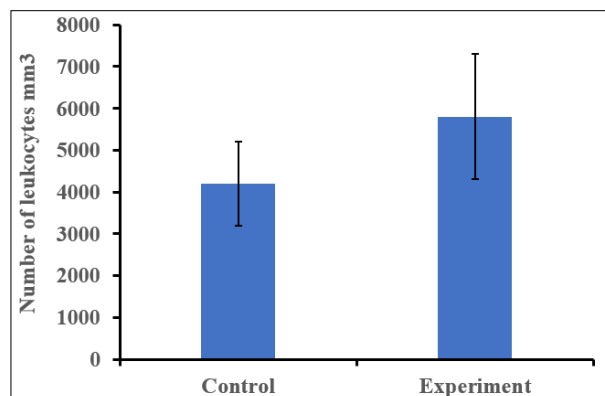
To assess the changes in proliferative activity, the colchicine mitotic index and the immunohistochemical analysis using proliferation marker protein Ki67 were used [5,9].

Data are expressed as mean SD. Student's t test was used for comparison among the different groups.  $P < 0.05$  was considered statistically significant.

### Results

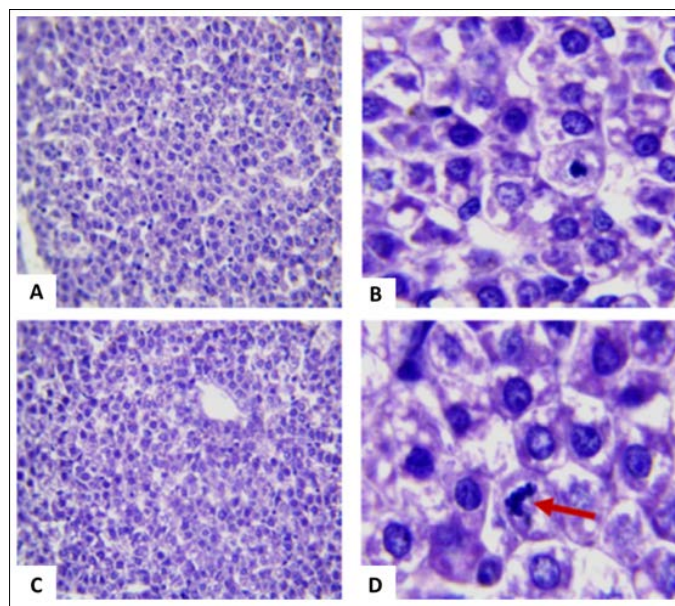
In order to introduce the hen's liver TPC into the body in a non-invasive way, we used the polyvinyl alcohol functional polymer developed under the leadership of Prof. Omar Mukbanian, Iv. Javakhishvili's Tbilisi State University, the department of Macromolecules. Initially, we investigated the possible negative (toxic) effects of the mentioned functional polymer on the experimental animals. For this purpose, we placed the polyvinyl alcohol functional polymer film (1.5 cm diameter) on the

abdominal surface of 7-day-old white rats. In order to attach the film to the surface of the skin, we periodically dripped distilled water on the film. After two hours, we determined the change in the leukocytes total number of the peripheral blood in the control and experimental animals (Figure 1).



**Figure 1:** The Influence of Polyvinyl Alcohol Functional Polymer on the Change of the Total Number of Leukocytes in the Peripheral Blood of 7-Day-Old Rats ( $p > 0.5$ )

It was determined that the leukocytes total number of the peripheral blood in the animals of the experimental group does not reliably differ from the corresponding indicators of the animals from the control group. At the same time, we evaluated the influence of the polyvinyl alcohol functional polymer on the changes of the liver tissue histoarchitectonics in both groups of animals.



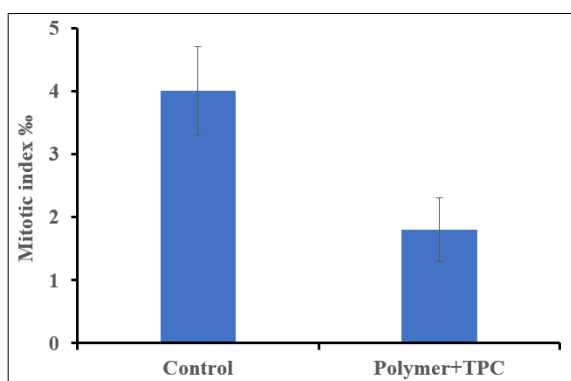
**Figure 2:** Liver Tissue Histoarchitectonics of the Adolescent Rats (H&E). Control (A- x400; B-x1000); Experiment (C -x100; d-x1000) (The Arrow Shows the Mitotic Figure)

The photomicrographs of the adolescent rats' liver paraffin sections, presented in Figure 2, show no visible changes in the liver histoarchitectonics of the experimental animals. In particular, there is no revealed infiltration characteristic of the inflammatory process and no increased sinusoidal diameter (Figure 2).

At the next stage of the research, the polyvinyl alcohol functional polymer film was used, into which TPC powder (200 µg) isolated from the liver of hens was immobilized. The animals were divided into two groups:

1. Control group – the intact adolescent rats
2. Experimental group – the adolescent rats, on the abdomen surface were placed the polymer with TPC (1.5 cm diameter). One hour later, the both groups' animals were injected with colchicine (1 µg/g). After two hours of the colchicine injection, the liver tissues were collected, fixed, and prepared for light microscopic examination.

From the presented diagram it can be seen that the colchicine mitotic index of animals in the experimental group decreases about 37% compared with the corresponding indicator of the control group (Figure 3).

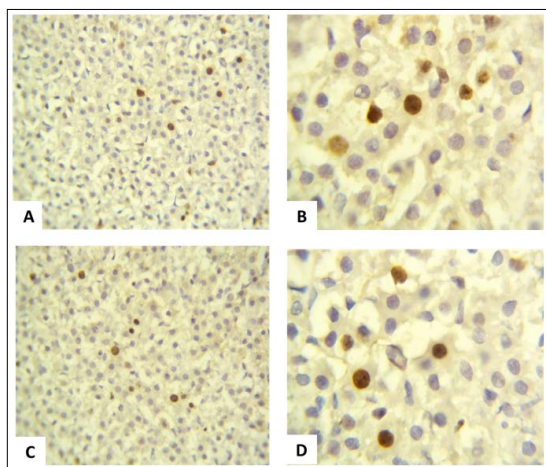


**Figure 3:** The Effect of the Hen's Liver TPC Immobilized into the Polyvinyl Alcohol Functional Polymer on the Proliferative Activity of the Homologous Tissue of White Adolescent Rats ( $p < 0.05$ )

We also assessed the effect of the TPC on the cell proliferation using antibodies against the proliferation marker protein Ki67 (immunohistochemistry).

Figure 4 represents immunohistochemical analysis of the liver tissue in the control and experimental animals.

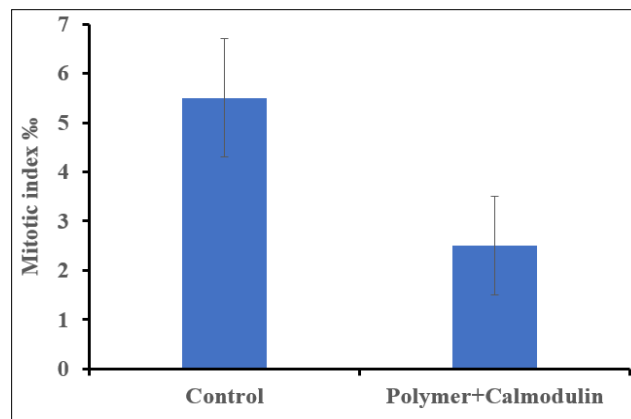
It was revealed that the ki67 positive cells increased in the number against the background of the decreased mitotic activity of hepatocytes in the animals of the experimental group.



**Figure 4:** Liver Tissue Histoarchitectonics of the Adolescent Rats (Immunohistochemistry, Ki67 Staining, Hematoxylin). Control (A – x400; B - x1000); Experiment (C - x400; D - x1000)

At the next stage of the study, the effect of the exogenous calmodulin, as active component of TPC, immobilized in the polyvinyl alcohol functional polymer, on the mitotic activity in the liver of adolescent rats (7 days of age) was assessed.

It can be seen from the presented histogram that the exogenous calmodulin is absorbed from the functional polymer film by the skin of adolescent rats, as like the thermostable protein complex, and inhibits the mitotic activity of the liver tissue about 40% (Figure 5).



**Figure 5:** Effects of Exogenous Calmodulin Immobilized into the Polyvinyl Alcohol Functional Polymer on the Mitotic Activity of the Adolescent Rats' Liver ( $p < 0.05$ )

### Discussion

We have established that the thermostable proteins complex of eu- and prokaryotic cells, which causes inhibition of mitotic activity by inhibition of transcription, is characterized by tissue specificity and does not show species specificity [3,10].

At the same time, it has been revealed that TPC of hemangioma does not have an inhibitory effect on the multiplication of heterotypic cells in proliferating tissues, which is explained by the different (minor) content of the low-molecular component (calmodulin) in the complex. This opinion was confirmed in the experiments in which a well-tested experimental model of hemangioma - the juvenile chicken bib was used [11,12].

The growth retardation of the bib was achieved through the injection of TPC obtained from the liver of adult hen [13,14].

Taking into account all the above, in the presented work we tried to determine whether the endogenous protein complex maintains its activity in case of non-invasive introduction into the body. For this purpose, a polyvinyl alcohol functional polymer was used. In order to rule out possible negative (toxic) effects of the polymer film on the morphofunctional activity of living cells, it was determined by the experiment that the polyvinyl alcohol functional polymer does not cause a change in the total number of leukocytes in the peripheral blood of adolescent rats for three hours. Also, there were no detected changes in the liver tissue, such as the lumen of the central vessels, as well as an excess of circulating cells in the liver tissue, which usually indicate the development of inflammation and/or other pathological processes. It follows from the obtained results that the single use of polyvinyl alcohol functional polymer does not have a negative effect on the peripheral blood and liver histoarchitectonics of adolescent rats.

**Citation:** Diana Dzidziguri, Elza Markarashvili, Irina Modebadze, Levan Rusishvili, Nino Kharashvili, et al. (2024) Development of a New Non-Invasive Approach to Treat of Infantile Hemangioma with Growth-Inhibiting Endogenous Factors (Non-Invasive Approach to Treat of Infantile Hemangioma with Endogenous Factors). *Progress in Medical Sciences*. PMS-1116.

Studies conducted to determine the feasibility of non-invasive delivery of a thermostable protein complex revealed a 37% reduction in the mitotic index of hepatocytes after exposure to TPC of the hen's liver immobilized into the functional polymer, and an increase in ki67 positive cells was noted in the same tissue by immunohistochemical analysis. This is explained by the high proliferative activity of the liver tissue of adolescent rats. Particularly, in response to suppressed mitotic activity of the proliferative tissue, the process of cell entry into the cell cycle was accelerated. A similar quantitative increase has also been described in the case of adult rat's TPC which has suppressive effect on the proliferation of homologous cells [5]. The obtained results indicate that chicken liver TPC pumped into the functional polymer is absorbed through the skin and reaches the liver, that is, it retains the ability to suppress the proliferative activity of the homologous tissue of adolescent rats. A similar effect was achieved on the mitotic activity of juvenile (7-day-old) rat livers by exposure to exogenous calmodulin immobilized into the polyvinyl alcohol functionalized polymer. Like a thermostable protein complex, exogenous calmodulin is released from the polymer film, absorbed from the skin of adolescent rats, and initiates inhibition of liver cell mitotic activity.

## Conclusion

From the analysis of the obtained results, it follows that:

- [1] The functional polymer of polyvinyl alcohol does not have a negative effect on the peripheral blood and the liver histoarchitectonics of the adolescent white rats;
- [2] The protein complex immobilized into a functional polymer and its active component, calmodulin, retains the ability to suppress the proliferative activity of the adolescent rat liver.
- [3] There is a possibility of therapeutic use of polyvinyl alcohol functional polymer film and exogenous calmodulin immobilized into it.

At the same time, in order to clarify the expediency of using the mentioned approach, it is necessary to conduct additional studies, including preclinical research.

## References

- [1] Novoa M, Baselga E, Beltran S, Giraldo L, Shahbaz A, et al. Interventions for infantile haemangiomas of the skin. *Cochrane Database Syst Rev*. 2018; 18: 4.
- [2] Chen Zhao-Yang, Qing-Nan Wang, Yang-Hui Zhu, Ling-Yan Zhou, Ting Xu, et al. Progress in the treatment of infantile hemangioma. *Ann Transl Med*. 2019; 7: 692.
- [3] Dzidziguri DV, Chigogidze TG, Managadze LG, Aslamazishvili TT, Kerkendzhia SM. Kidney protein complexes that inhibit gene expression in the nuclei of homotypic cells. *Georgian Med News*. 2007; 50-53.
- [4] Vadachkoria Z, L Dzidziguri, E Bakuradze, I Modebadze, N Oganezovi, et al. Comparative study of growth inhibitory protein complex derived from different forms of children hemangioma. *Georgian medical news*. 2015; 9: 73-77.
- [5] Dzidziguri Diana, Irina Modebadze, Ekaterine Bakuradze, Giorgi Mosidze, Manana Berulava. Properties of the thermostable protein complex of rat brain inhibiting the cell proliferation. *Cell J (Yakhteh)*. 2018; 19: 552-558.
- [6] Bullow WS, Hewett C, Laurence E. The epidermal chalone: a preliminary attempt at isolation. *Exp.Cell Res*. 1964; 36: 192-200.
- [7] Mukbaniani O, J Aneli, T Tatrishvili, E Markarashvili, M Chigvinadze, et al. Synthesis of cross-linked comb-type polysiloxane for polymer electrolyte membranes. *E-polymer*. 2012; 89: 1-14.
- [8] Aneli J, O Mukbaniani, E Markarashvili, G Zaikov, E Klodzinska. Polymer composites on the basis of epoxy resin with mineral fillers modified by tetraethoxysilane. *Chemistry and Chemical Technology*. 2013; 7: 141-145.
- [9] Cardiff RD, Miller CH, Munn RJ. *Manual Hematoxylin and Eosin Staining of Mouse Tissue Sections*. Cold Spring Harbor Protocols. 2014; 655-658.
- [10] Giorgobiani N, Dzidziguri D, Rukhadze M, Rusishvili L, Tumanishvili G. Possible role of endogenous growth inhibitors in regeneration of organs: searching for new approaches. *cel biol intern*. 2005; 29: 1047-1049.
- [11] Ritter EJ. The chicken comb and wattle as an experimental model for the therapy of hemangiomas. Preliminary laser studies. *Life Sci*. 1966; 5: 1903-1910.
- [12] Anvari B, BS Tanenbaum, TE Milner, W Hoffman, S Said, et al. Cryogen Spray Cooling for Spatially Selective Photocoagulation: a Feasibility Study with Potential Application for Treatment of Hemangiomas. *Proc. SPIE*. 1996; 2922: 29-37.
- [13] Ghibradze G, Z Vadachkoria, L Dzidziguri, M Mikadze, I Modebadze, et al. Development of New Approaches to the Treatment of Hemangiomas in Experiment. *Georgian Medical News*. 2022; 323: 112-115.
- [14] Gunia Veronika, Mamuka Kordzadze, Alexandre Kordzadze, Giorgi Ghibradze, Diana Dzidziguri. Experimental evidence of the possibility of delivery the growth inhibitory factor immobilized in functional polymer. Tenth international medical congress of seemf. Sofia, Bulgaria. 2019; 109-110.