The Effect of Polyscias filicifolia Bailey Biomass Tincture on the Protein Synthesis Process in the Heterogeneous System From the Isolated Pig Heart

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Key Words: anoxia; protein synthesis; pig heart; Polyscias filicifolia Bailey.

Summary. Background and Objective. An insufficient supply of oxygen to the heart influences the process of protein synthesis. The aim of this study was to determine the effect of the Polyscias filicifolia Bailey biomass tincture on the protein synthesis process in a heterogeneous translation system from the isolated pig heart.

Materials and Methods. The effect of anoxia was evaluated after 20- and 90-minute anoxia. With the aim to determine the effect of Polyscias, the pig hearts were perfused with a buffer containing the Polyscias filicifolia Bailey biomass tincture. To determine the rate and the level of translation, the incorporation of [¹⁴C]-leucine into translational products in a cell-free system was measured.

Results. The protein synthesis level decreased by 23%–42% when the translation system containing cytosol from the anoxic heart was used. When the translation system containing a ribosomal fraction after 20-minutes anoxia was used, the protein synthesis level was the same as in the control. In the case of 90-minute anoxia, it decreased by 16%. The protein synthesis rate and the level in the translation system containing cytosol from the heart after 20-minute anoxic perfusion with the buffer containing Polyscias was the same as in the control.

Conclusions. A decrease in the protein synthesis rate and the level after 20-minute anoxia was determined by changes in cytosol. On the other hand, 90-minute anoxia caused changes in cytosol and the ribosomal fraction. The Polyscias filicifolia Bailey biomass tincture restored the protein synthesis process acting on the components of the translation system in cytosol and the ribosomal fraction.

Introduction
An insufficient supply of oxygen to tissues and organs is the main factor influencing metabolic processes in various cells and tissues (1–3). The heart is an exceptionally important organ for the work of the body and exceptionally sensitive to the lack of oxygen (ischemia, hypoxia, or anoxia). It has been determined that 20-minute oxygen deprivation induces reversible histochemical and functional changes in the myocardium, and 20- to 40-minute oxygen deprivation induces irreversible changes in the myocardium (4). The lack of oxygen influences protein synthesis at different levels of this process. It may be associated with the rate of energy production (5), RNA transcription regulation (6), aminoacyl-tRNA formation (7, 8), and regulation of mRNA translation (9–11).

Biologically active substances such as adaptogens have been used in medicine to prevent and cure various pathologies for a long time. It has been determined that Panax ginseng C. A. Mey and other plants of the Araliaceae family are characterized by anti-inflammatory and antioxidant activities (12, 13). These plants act through the endocrine system and regulate stress reactions (14). It has been determined that Panax ginseng and other plants of the Araliaceae family protect against ischemia and anoxia (2, 14, 15). One of them is Polyscias filicifolia Bailey. Polyscias filicifolia Bailey biomass, in the same way as the Panax ginseng root, contains triterpenic saponins and protects from stress reactions and disturbances of oxygen deprivation (7, 14, 16–19). It is worth noting that the alcohol extract of Polyscias filicifolia biomass does not show cytotoxic and genotoxic activity. It allows hoping that Polyscias filicifolia could be used as a substance showing therapeutic properties (20).

Despite the fact that we have determined the protective effect of the Polyscias filicifolia Bailey biomass tincture on the protein synthesis process in the homogeneous protein synthesis system in a pig heart under anoxia (16), the definite mechanisms of the protective effect of the Polyscias filicifolia Bailey biomass tincture on some components of the translation system...
system under anoxia are not particularly known.

The aim of this study was to determine the effect of the *Polyscias filicifolia* Bailey biomass tincture on the protein synthesis process in the heterogeneous protein synthesis system from the isolated pig heart.

**Material and Methods**

**Study Subjects and Protocol.** The experiments were carried out on isolated pig hearts weighing 100–150 g. The pig hearts were obtained from a slaughterhouse. Preparation, control, and anoxic perfusion were performed immediately after slaughter.

**Model of Perfusion and Anoxia Preparation.** The hearts were perfused according to the modified method of Langendorff (21), using an artificial blood circulation apparatus. Anoxia was performed by the perfusion of the isolated pig heart with the Krebs-Henseleit bicarbonate buffer saturated with a gas mixture (95% N₂ and 5% CO₂). The control hearts were perfused with the same buffer saturated with the gas mixture (95% O₂ and 5% CO₂).

**Experimental Groups.** The hearts were distributed into groups randomly. The effect of 20-minute anoxia was evaluated after 20 minutes of anoxic perfusion. The hearts in the other group were perfused for 90 minutes under anoxic conditions. These durations were chosen because the 20-minute anoxia caused reversible biochemical changes in the myocardium, whereas the 90-minute anoxia caused irreversible changes. With the aim to determine an effect of *Polyscias filicifolia* Bailey biomass, the pig heart was perfused under normoxic and anoxic conditions with a buffer containing the tincture of *Polyscias filicifolia* Bailey (0.5 mL tincture/1000 mL buffer).

*Polyscias filicifolia* Bailey biomass was obtained from Dr. V. A. Kunakh, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine. The tincture of *Polyscias filicifolia* Bailey was prepared according to the requirements for the preparations of tinctures (16).

**Preparation of Ribosomes and Cytosol.** The total preparation of ribosomes was isolated from the pig myocardium according to Wettstein et al. (22). A fraction of postribosomal supernatant (cytosol) was made by the 20-minute centrifugation of a pig heart homogenate at 30 000g. The obtained solution was filtrated and centrifuged for 120 minutes at 105 000g. The obtained supernatant was purified by chromatography on the Sephadex G-15 column (Sigma-Aldrich).

**Investigation of Protein Synthesis Process.** To determine the intensity of the protein synthesis, the incorporation of [¹⁴C]-leucine into translational products in a cell-free translation system was measured. The cell-free translation system (100 μL) contained 50-mM Tris-HCl (pH 7.6), 5-mM MgCl₂, 100-mM KCl, 1-mM dithiothreitol, 0.25-mM ATP, 0.25-mM GTP, 3-μg creatine phosphokinase, 10-mM creatine phosphate, 0.025-mM [¹⁴C]-leucine, 0.025-mM nonradioactive amino acids (except leucine), and amount of cytosolic or ribosomal fraction equivalent to 0.25 unit of optical density (A₂₆₀₋₃₄₀).

The investigation of protein synthesis kinetics showed that the maximum level of translation could be reached after the incubation of the reaction mixture for 60 minutes at 37°C. In order to determine the rate of translation, the incubation should continue for 15 minutes. Based on these experiments, the reaction mixture was incubated for 60 minutes at 37°C for the measurement of the protein synthesis level and for 15 minutes at 37°C for the measurement of the protein synthesis rate. The reaction was stopped by adding 0.1 M KOH (0.5 mL). TRNA deacylation was performed by heating the preparations for 20 minutes in a water bath. The reaction was stopped by adding 10% trichloracetic acid (1.0 mL). The samples were left in ice for 1 hour. The precipitate was collected on nitrocellulose filters and washed with 5% trichloracetic acid. The radioactivity was measured in a Delta 300 liquid scintillation counter.

The protein synthesis rate and level in the cell-free translation system was evaluated according to [¹⁴C]-leucine incorporation into an endogenous mRNA translation product. All the results were expressed as the mean ± standard error of the mean. The Student t test was used for comparisons. The statistical significance was set at *P*<0.05.

**Results**

The heterogeneous cell-free translation system was used in 4 groups (Figs. 1 and 2): 1) the translation system containing cytosol and a ribosomal fraction from the control heart; 2) the translation system containing cytosol from the control heart and a ribosomal fraction from the anoxic heart; 3) translation system containing cytosol from the anoxic heart and a ribosomal fraction from the control heart; and 4) the translation system containing cytosol and a ribosomal fraction from the anoxic heart.

Results in Fig. 1 show the rate and the level of protein synthesis in the cell-free translation system from the pig heart after 20 minutes of anoxic perfusion. The protein synthesis rate decreased by 28% and the level by 19% in the cell-free system containing cytosol and the ribosomal fraction from the anoxic heart as compared with the control heart. Analogous results were observed when the cell-free system contained cytosol from the anoxic heart and the ribosomal fraction from the control heart (22% and 23%, respectively). When the translation system containing cytosol from the control heart and the ribosomal fraction from the anoxic heart was used, the results were the same as in the control heart.
The incubation time for the measurement of the \([^{14}C]\)-leucine incorporation rate was 15 minutes (1); the incubation time for the measurement of the \([^{14}C]\)-leucine incorporation level was 60 minutes (2). The data represent the results of 6–8 separate experiments.

*P<0.05, comparing the control and experimental groups.

In the case of the 90-minute anoxia (Fig. 2), the rate and the level of protein synthesis diminished by 48% and 45%, respectively, in comparison with the control heart when cytosol and the ribosomal fraction from the anoxic heart were used. Analogous results were observed when the cell-free system containing ribosomal fractions from the control heart and cytosol from the anoxic heart was used (46% and 42%, respectively). When the cell-free system containing cytosol from the control heart and the ribosomal fraction from the anoxic heart was used, the rate and the level of protein synthesis diminished by 23% and 16%, respectively, in contradiction to the results observed after 20 minutes of anoxia.

The effect of the *Polyscias filicifolia* Bailey tincture on the rate and the level of protein synthesis in the heterogeneous cell-free translation system from the control and anoxic pig hearts was studied. A possible influence of ethanol on the rate and the level of protein synthesis in the cell-free system was studied by adding ethanol into the perfusion buffer. The concentration of ethanol was the same as in the tincture of *Polyscias filicifolia* Bailey. No statistically significant differences were obtained after the perfusion with the buffer containing ethanol under normoxic and anoxic conditions.

With the aim of determining which component of a cell-free translation system (ribosomal fraction or cytosol) was influenced with *Polyscias filicifolia* Bailey under anoxic conditions, the heterogeneous cell-free translation system was used. The cell-free translation system contained a ribosomal fraction from the control heart and the following (Figs. 3 and 4): 1) cytosol from the control heart; 2) cytosol from the anoxic heart; and 3) cytosol from the *Polyscias*-treated anoxic heart.

Another group of the cell-free protein synthesis system was a set of cytosol from the control heart and the following (Figs. 5 and 6): 1) a ribosomal fraction from the control heart; 2) a ribosomal fraction from the anoxic heart; and 3) a ribosomal fraction from the *Polyscias*-treated anoxic heart.

The results in Figs. 3 and 4 show that the rate and the level of protein synthesis in the cell-free translation system containing the ribosomal fraction from the control heart and cytosol from the anoxic heart significantly diminished in the cases of 20- and 90-minute anoxia in comparison with the control heart. In the cell-free translation system including cytosol from the heart after 20 minutes of anoxic perfusion with the buffer containing the *Polyscias filicifolia* Bailey biomass tincture and the ribosomal fraction from the control heart, the rate and the level of protein synthesis reached the control values (Fig. 3).

In the cell-free translation system containing cytosol from the heart after 90 minutes of anoxic perfusion with the buffer containing the *Polyscias filicifolia* Bailey tincture and the ribosomal fraction from the control heart, the rate and the level of protein synthesis did not reach the control values and were lower than in the control (Fig. 4).

*Fig. 1. The incorporation of \([^{14}C]\)-leucine into translation products in the cell-free protein synthesis system from the control pig heart and the heart after 20 minutes of anoxia (pmol per 1 unit of optical density, \(A_{260-320}\) ribosomal fraction).

The experiments were performed as described in Fig. 1.

*P<0.05, comparing the control and experimental groups.*
The results in Fig. 5 show that in the cell-free translation system containing cytosol from the control heart and the ribosomal fraction from the control heart, anoxic heart, or anoxic heart treated with the Polyscias filicifolia Bailey tincture in the case of the 20-minute anoxic perfusion, there were no significant differences in the rate and the level of protein synthesis observed.

In the case of 90-minute anoxia in the cell-free translation system containing cytosol from the control heart and the ribosomal fraction from the anoxic heart, the rate and the level of protein synthesis diminished by 25% and 16%, respectively (Fig. 6). When there was the cell-free translation system containing cytosol from the control heart and the ribosomal fraction from the anoxic heart treated with the Polyscias filicifolia Bailey tincture, the rate and the level of protein synthesis diminished by 20% and 14%, respectively (Fig. 6).

The experiments were performed as described in Fig. 5. *P<0.05, comparing the control and experimental groups.
Polyscias filicifolia Bailey, the rate and the level of protein synthesis decreased by 12% and 10%, respectively, in comparison with the control heart. There were no significant differences in the rate and the level of protein synthesis between the cell-free translation system containing the ribosomal fraction from the anoxic heart and the system containing the ribosomal fraction from the anoxic heart treated with the Polyscias filicifolia Bailey tincture.

**Discussion**

It was shown that the rate and the level of protein synthesis in the cell-free translation system significantly decreased as compared with the control heart when the heterogeneous cell-free protein synthesis system containing cytosol from the heart after 20- and 90-minute anoxic perfusion and the ribosomal fraction from the control heart was used.

When the ribosomal fraction from the heart after 90 minutes of anoxia and cytosol from the control heart were used, the rate and the level of protein synthesis diminished, too. However, the decrease was lower than in the case of the cell-free translation system containing cytosol from the anoxic heart.

It was determined that the Polyscias filicifolia Bailey tincture restored the rate and the level of protein synthesis in the case of 20-minute anoxia. In the case of 90-minute anoxia, it showed only a partial protective effect on the protein synthesis process.

The obtained results showed that on the one hand, the rate and the level of protein synthesis in the cell-free translation system under oxygen deprivation were related to the changes in cytosol and the ribosomal fraction. On the other hand, the changes in cytosol have caused a major decrease in the rate and the level of protein synthesis. As reported earlier, a decrease in protein synthesis intensity under oxygen deprivation was related to the changes in the activity of aminoacyl-tRNA synthetases and tRNA methyltransferases (23), tRNA and aminoacyl-tRNA synthetases in the rabbit liver (8), and tRNA and aminoacyl-tRNA synthetases in the pig myocardium (7, 17). The decrease in the rate and the level of protein synthesis in the cell-free translation system from the pig heart after 20 and 90 minutes of anoxia was similar to the decrease in the activity of tRNA and aminoacyl-tRNA synthetases under the same conditions. Consequently, we can conclude that one of the causes influencing the protein synthesis process in the cell-free translation system under anoxia may be by the changes in the activity of tRNA and aminoacyl-tRNA synthetases under anoxia.

It has been known that experimental myocardial infarction causes a reduction in the ribosome production and ribosomal RNA amount in the rabbit liver (18). Under total ischemia of the myocardium (autolysis), the ribosome activity decreases, too (24). These data may explain a decrease in the rate and the level of protein synthesis in the cell-free translation system containing the ribosomal fraction from the heart after 90-minute anoxia.

The obtained results allow us assume that the disturbances in the protein synthesis system in the pig heart under anoxia may be associated with the changes in the activity of the components of the translation system. The protective effect of the Polyscias filicifolia Bailey biomass tincture may be related with the action of the tincture on ribosomes and components of the translation system in cytosol. The data reported in a study by Liekis et al. (18) indicated that the content of ribosomes and the amount of RNA in the rabbit liver after the reproduction of experimental myocardial infarction significantly decreased. However, the use of Polyscias filicifolia Bailey biomass leads to a partial restoration of the RNA content. At the same time, a change in the content of ribosomes in the rabbit liver should affect the level of protein synthesis in this organ.

As reported earlier, the preparations of Polyscias filicifolia Bailey biomass have a protective effect on the activity of tRNA and aminoacyl-tRNA synthetases in the rabbit liver under experimental myocardial infarction (19) and in the pig heart under anoxic perfusion (7, 17). In the case of the 20- and 90-minute anoxic perfusion, the protective effect of the Polyscias filicifolia Bailey tincture on the protein synthesis process was analogous to the protective effect on the activity of tRNA and aminoacyl-tRNA synthetases. Consequently, we can conclude that the protein synthesis process during anoxia may be protected by the effect of the Polyscias filicifolia Bailey biomass tincture on the activity of tRNA and aminoacyl-tRNA synthetases. It is known that the activity of tRNA in the pig heart is regulated by the formation of inactive conformers under anoxic conditions. The activity of aminoacyl-tRNA synthetases is regulated by changing the activity of inorganic pyrophosphatase under anoxia (17). The use of the Polyscias filicifolia Bailey biomass tincture can protect the heart from these changes.

**Conclusions**

The diminished rate and level of protein synthesis in the heterogeneous cell-free protein synthesis system after 20 and 90 minutes of anoxic perfusion were related to the changes in cytosol and ribosomes. In the case of anoxic perfusion with buffer containing the Polyscias filicifolia Bailey biomass tincture, the protective effect of Polyscias filicifolia Bailey on protein synthesis was observed. This effect was determined by its influence on the activities of components of cytosol and ribosomes.

**Statement of Conflict of Interest**

The authors state no conflict of interest.
References
1. Lui L, Cash TP, Jones RG, Keith B, Thomson CB, Simon MC. Hypoxia-induced energy stress regulates mRNA translation and cell growth. Mol Cell 2006;21:521-31
2. Park H, Kim H, Ha E, Yoon S, Kim MJ, Hong M, et al. Panax ginseng increases hypoxia-induced downregulated cellular response related genes in human neuroblastoma cells. SK-N-MC. Neurol Res 2007;Suppl 1:S78-87.
3. Zhu L, Wang Q, Zhang L, Fang Z, Zhao F, Lv Z, et al. Hypoxia induces PGC-1α expression and mitochondrial biogenesis in the myocardium of TOF patients. Cell Res 2010;20:676-87.
4. Humphrey, SM, Cartner LA, Holliss DG. Critical early metabolic changes associated with myocardial recovery of failure after total ischemia in the rat heart. Basic Res Cardiol 1987;82:304-16.
5. Casey TM, Pakay JL, Guppy M, Arthur PG. Hypoxia causes downregulation of protein and RNA synthesis in noncontracting mammalian cardiomyocytes. Circ Res 2002;90:777-83.
6. Ernens I, Goodfellow SJ, Innes F, Kenneth NS, Derblay LE, White RJ, et al. Hypoxic stress suppresses RNA polymerase III recruitment and tRNA gene transcription in cardiomyocytes. Nucleic Acids Res 2006;34:286-94.
7. Kalauskas A, Radovičius H, Vieželiene D. Effect of Polyscias filicifolia Bailey tincture on tRNAleu and leucyl-tRNA synthetase activity in isolated pig heart. Biologija (Vilnius) 2006;47:2-5.
8. Radovičius H, Burneckienė J. Seasonal differences in activities of rabbit liver tRNA and aminoacyl-tRNA synthetases specific for valine and arginine under myocardial ischemia. Medicina (Kaunas) 2006;42:225-30.
9. Uniackie J, Holterman CE, Lachance G, Franovic A, Jacob MD, Fabian MR, et al. An oxygen-regulated switch in the protein synthesis machinery. Nature 2012;486:126-9.
10. Iacobas D, Fan C, Iacobas S, Haddad GG. Integrated transcriptomic response to cardiac chronic hypoxia: translation regulators and response to stress in cell survival. Funct Integr Genomics 2008;8:265-75
11. Kanatous SB, Mammen PPA, Rosenberg PB, Martin CM, White MD, Dimaiho JM, et al. Hypoxia reprograms calcium signaling and regulates myoglobin expression. Am J Physiol Cell 2009;296:C393-402.
12. Džukar CM, Sheela S, Sandhya S, Vinod KR, Pillai NR, Rao SB. Anti-inflammatory and antioxidant activities of Polyscias filicifolia saponins. Der Pharmacia Lettre 2010;2:41-7.
13. Masteikova R, Muselik J, Bernatoniene J, Bernatoniene R. Antioxidative activity of ginkgo, Echinacea and Ginseng tinctures. Medicina (Kaunas) 2007;43:306-9.
14. Trilis G, Davydov VV, Slepian LI. Vlijanije preparatov kul-tury tkanej Panax Ginseng C. A Mey i Polyscias filicifolia Bailey (Araliaceae) na tetscheniie poststressornyck peacii. (The influence of preparations of cultured tissues of Panax ginseng C.A. Mey and Polyscias filicifolia Bailey (Araliace-ae) on post stress processes.) Psicofarmacol Biol Narkol 2005;5:1071-80.
15. Li XT, Chen R, Jin LM, Chen HY. Regulation on energy metabolism and protection on mitochondria of Panax ginseng polysaccharide. Am J Chin Med 2009;37:1139-52.
16. Kalauskas A, Vieželiene D. Effect of Polyscias filicifolia Bailey biomass on protein synthesis process in isolated pig heart. Medicina (Kaunas) 2004;40:991-6.
17. Kalauskas A, Radovičius H, Vieželiene D, Lažauskas R. Effect of anoxia an Polyscias filicifolia Bailey biomass tincture on the activity of tRNA and aminoacyl-tRNA synthetases in isolated pig heart. Medicina (Kaunas) 2009;45:486-92.
18. Lekis AV, Mashanauskas TK, Ivanov LL, Lukoshivichyus LY, Kunakh VA, Kovalenko MI, et al. Influence of cultured Polyscias cells on protein biosynthesis in the rabbit liver. Pharm Chem J 1988;22:643-7.
19. Slavinskiene RJ, Lukosevicius IJ, Kunach BA, Slepian LI, Kovalenko MI, Ivanov LL. Vilijanije biomass kultivuiruem-yck kletok Polyscias filicifolia Bailey na aktivnostj tPHK i aminoacil-tPHK-sintetaz petcheni krolokov. (Effect of bio-mass of cultivated cells Polyscias filicifolia Bailey on activity of tRNA and aminoacyl-tRNA synthetases from rabbit liver.) Biopolimery in kletka 1986;2(3):152-3.
20. Marczewska J, Karwica E, Drozd J, Anuszewskal E, Slwin-aska A Nosov A, et al. Assessment of cytotoxic and geno-toxic activity of alcohol extract of Polyscias filicifolia shoot, leaf, cell biomass of suspension culture and saponin fraction. Acta Pol Pharm 2011;68:703-10.
21. Morgan HE, Henderson MJ, Regen DM, Park CR. Regulation of glucose uptake in muscle. I. The effect of insulin and anoxia and glucose transport and phosphorylation in the isolated perfused heart of normal rats. J Biol Chem 1971;246:253-61.
22. Wettstein FD, Staechelin T, Noll H. Ribosomal aggregate engaged in protein synthesis characterization of the ergosomes. Nature 1963;197:430-7.
23. Vieželiene D, Ivanov LL, Radovičius H, Praškevičius A. The activity and aggregate of rabbit liver aminoacyl-tRNA synthetases and tRNA methyltransferases under myocardial ischemia. Biologija (Vilnius) 1995;5:283-95.
24. Praškevičius A, Lukoševicius I, Ivanov LL, Sadauskienė I, Stapulionis R. Balties biologijos (kaunas) 2001;37:1488-93.

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